# BREEDING SWITCHGRASS FOR RESISTANCE TO BIPOLARIS DISEASES

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**BREEDING SWITCHGRASS FOR RESISTANCE TO BIPOLARIS DISEASES**

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Switchgrass (*Panicum virgatum* L.), a perennial C4 biomass crop native to North America, suffers from a reduction in germination due to Bipolaris seed rot (BSR) and yield reduction from Bipolaris leaf spot (BLS), both caused by a necrotrophic ascomycete fungus named *Bipolaris oryzae* (Breda de Haan) Shoemaker. To manage the diseases under economically competitive conditions, breeding switchgrass for resistance and silicon amendment were two potential approaches. Screening methods in a greenhouse were established for BSR in seeds and BLS in 4-week-old seedlings. Half-sib progenies were used to estimate narrow-sense heritability of resistance to the two diseases. Moderate heritability estimates of resistance to BSR suggested successful gain from selection, whereas non-significant heritability of resistance to BLS suggested no progress from selection. Such heritabilities accurately predicted the result from two cycles of recurrent phenotypic selection in 'Cave-in-Rock' and 'Shelter'. The progress of resistance to BSR was more than 50% cycle$^{-1}$ in both cultivars whereas the progress of resistance to BLS was not significant. Such difference between resistances to BSR and BLS resulted in no correlation between the disease resistances. To dissect the resistance to BLS, genome-wide association was conducted in the Northern Association Panel. The BLS evaluation was conducted in 479 mature switchgrass plants via field evaluation, detached leaf assay, and leaf disk

assay. Multi-trait Genome-wide association studies (GWAS) from different phenotype combinations in four subgroups revealed potential resistance genes associated with 18 markers on chromosomes 1b, 2a, 2b, 3a, 3b, 5a, 5b, 6a, 7a, 8a, 9a, and 9b explaining phenotypic variances of 6.32 to 26.72%. Within linkage disequilibrium of 20 kb, there are some potential resistance genes including genes encoding Myb, cytochrome P450, isocitrate lyase, E3 ubiquitin-protein, etc. These markers can be used in genomics-assisted breeding in the future. Besides breeding, silicon amendment has also explored the potential based on the effectiveness against BLS in rice. However, for switchgrass in a greenhouse, silicon amendment (either incorporated into the potting mix or foliar drenches) showed no significant effect BSR and BLS, suggesting more studies are needed on field application and long-term effects of silicon.

## BIOGRAPHICAL SKETCH

Kittikun (Chris/Ob) Songsomboon was born July 24, 1989 in Muang, Ratchaburi and grew up in Sampran, Nakhon Pathom, THAILAND. He earned his Bachelor of Science in Biology (Botany) with Honors from Silpakorn University in 2012. After one year as a research trainee, he joined the doctoral program in Plant Breeding and Genetics at Cornell University in 2013.

Dr. Songsomboon has received numerous honors and awards since his undergraduate education. He was granted a scholarship from the Development and Promotion of Science and Technology Talents Project during his undergraduate degree until his doctoral degree abroad.

While pursuing his doctoral degree, Dr. Songsomboon worked as a research assistant and graduate teaching assistant for Dr. Donald R. Viands – his principal investigator. He was active in the Plant Breeding Graduate Assembly, Synapsis, serving as the Graduate & Professional Student Assembly (GPSA) Field Representative.

Dr. Songsomboon's dissertation, *Breeding Switchgrass for Resistance to Bipolaris diseases*, was supervised by Dr. Donald R. Viands.

After completing his doctoral degree, Dr. Songsomboon planned to pursue his career in academia as a plant breeder in Thailand.

This dissertation is dedicated to my family, especially my mother, Nimnuan Songsomboon. Without her unconditional understanding, warm-hearted sacrifice, and ultimate love, this achievement could not have been possible.

I deeply appreciate my life partner, Mingtao Wu, for the love, patience, support, and delicious meals all the way through graduate school.

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# SWITCHGRASS-BIPOLARIS ORYZAE PATHOSYSTEM

## Introduction

As the world's energy demand is expected to increase from 12 billion tons of oil equivalent in 2009 to 17 billion t.o.e. by 2035, and likewise carbon-dioxide emissions to increase from 29 gigatons per year to 36 gigatons per year, biorenewable energy has been considered as a sustainable solution to energy insufficiency (Chu and Majumdar, 2012). Biomass is considered one of the most practical biorenewable energy sources. When considering high yield and greenhouse gas (GHG) emissions across other biomass crop candidates, switchgrass (*Panicum virgatum* L.) showed promising features of saving GHG (14 tons $CO_2$ equivalent·$ha^{-1}$) and high yield of 17 dry ton·$ha^{-1}$ (Figure 1.1) (Cherubini et al., 2009).

Such promising yield cannot be maintained under threats from destructive diseases. *Bipolaris oryzae* (Breda de Haan) Shoemaker (*B. oryzae*) is one of the prominent ascomycete fungi, causing Bipolaris seed rot that reduced seed germination up to 70%, and Bipolaris leaf spot that reduced yield up to 50% (Fajolu, 2012). The agronomic practices for switchgrass – propagation by seeds, perennial production, low nutrient input in marginal land – intensify Bipolaris diseases in switchgrass because of no disruption from agricultural practices (such as crop rotation).

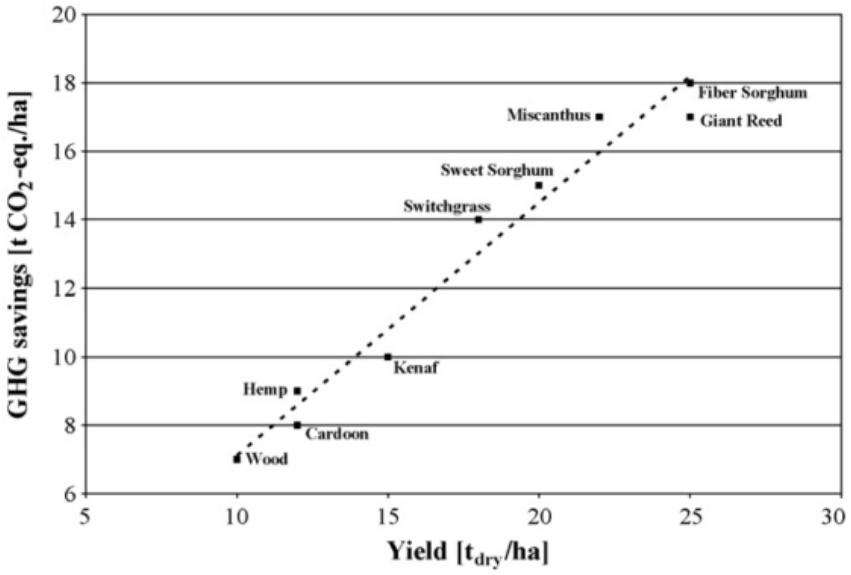


Figure 1.1. Greenhouse gas savings per hectare in lignocellulosic crop yields in electricity (Cherubini et al., 2009).

## Switchgrass

### Taxonomy, characteristics, production, and challenges

Switchgrass (*Panicum virgatum* L.) is a warm-season (C4) perennial grass native to North America. The genus *Panicum* is a member of the grass family (*Poaceae*) and subfamily Panicoideae. Based on sequence analysis of the conserved nuclear *Acc-1* gene encoding acetyl-CoA-carboxylase, the tribe Paniceae of switchgrass diverged about 23 million years ago from the tribe Maydeae of maize (*Zea mays*) (Huang et al., 2003). Switchgrass is widely dispersed from Central America to southern Canada and across the east of the Rocky Mountains (Hitchcock, 1935). Such an enormous distribution is the result of diverse genetic variation within the species (Parrish and Fike, 2005). The broad categorization based on the morphology can group switchgrass into two ecotypes including upland and lowland types (Figure 1.2)

(Porter, 1966; Casler et al., 2011). Upland types are generally well-adapted to higher latitudes with winter hardiness and more mesic areas. In contrast, lowland types are suitable for lower latitudes and sensitive to water stress. Besides the location, the lowland ecotypes are normally taller and coarser than the upland types. The lowland stems are also thicker; the leaves are longer, wider, and more bluish-green; and the panicles are bigger (Porter, 1966; Casler, 2005) (Figure 1.3). Another morphologically distinct characteristic is the origin of the shoots. Lowland shoots originate only from buds on rhizomes, whereas upland shoots are from more active rhizomes and the basal nodes of culms produced in the previous year, which is similar to cereals and other grasses from tillers (Porter, 1966). The last morphological difference between upland and lowland is that the lowland root diameters are larger, but its root internodes and root length are shorter than upland ecotypes.

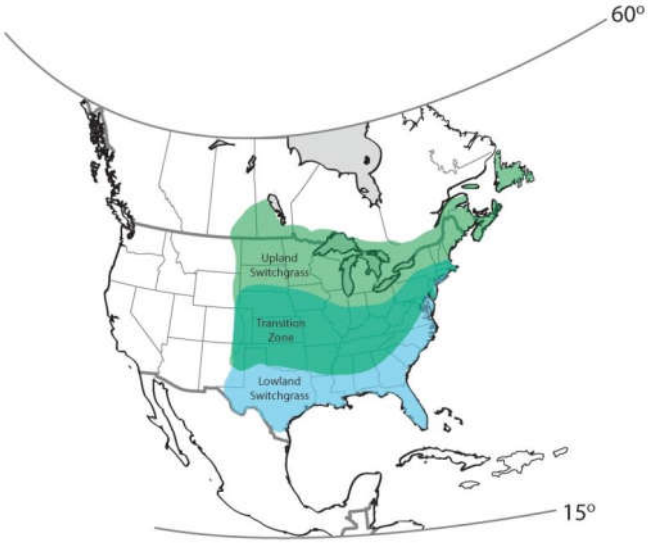


Figure 1.2. The distribution of lowland and upland ecotypes across North America (Casler et al., 2011).

Figure 1.3. Overall morphological comparison between upland (left) and lowland (right) ecotypes (NEWBio, 2016).

The genetic variation in switchgrass can be seen from the polyploidization ranging from tetraploid (4X = 36) to dodecaploid (12X = 108) (Lu et al., 1998). Lowland ecotypes are generally tetraploid, whereas the upland ecotypes are mostly octaploid with some exceptions in various populations. Genotype by sequencing (GBS) revealed the disomic segregation in tetraploid switchgrass, whereas octaploid switchgrass behaves like an autotetraploid (Lu et al., 2013). The disomic inheritance of tetraploid switchgrass simplifies future genetic research. They also confirmed the clear genetic distribution patterns of isolation-by-distance and isolation-by-ploidy. The phylogenetic analysis also suggested the migration of switchgrass from south to north. Interestingly, tetraploid upland arose from octaploid upland via apomixis.

Initially, switchgrass was introduced as a forage crop (Balasko et al., 1984). It was also used for various purposes such as natural conservation for wildlife habitat,

soil surface erosion control, and broad ornamental use in the landscaping industry (Wolf and Fiske, 1995; Parrish and Fike, 2005; Max et al., 2012). In the last decade, switchgrass has been studied as a source of bioenergy (McLaughlin and Kszos, 2005a). Switchgrass showed to be a sustainable resource by producing renewable energy five times higher than energy input (Schmer et al., 2008). Moreover, switchgrass can provide long-term (5 years) biomass at approximately 14 Mg $ha^{-1}$ without yield decline (Fike et al., 2006). Despite the high yield, planting switchgrass has its challenge in the establishment in the field, including small seed size, high seed dormancy, slow germination, and poor seedling vigor (Hsu and Nelson, 1986; Aiken and Springer, 1995; Hintz et al., 1998; Evers and Parsons, 2003). To handle these agronomic challenges, research has been focusing on reducing seed dormancy, improving the field establishment, and biomass yield (Tischler et al., 1994; Hintz et al., 1998; Vogel and Mitchell, 2008).

In addition to efforts on agronomic problems, research on improving energy conversion efficiency has also been supported, such as improving In Vitro Dry Matter Digestibility (IVDMD) and ethanol yield via breeding (Vogel et al., 2002; Fu et al., 2011). Switchgrass is an open pollinated crop and has high self-incompatibility with both pre- and postfertilization incompatibility mechanisms (Martínez-Reyna and Vogel, 2002). Additionally, owing to its recent domestication for monoculture in the 1970s (Balasko and Smith, 1971), switchgrass remains genetically diverse within cultivated populations, which could enable more advanced selection in a breeding program. Recurrent phenotypic selection is considered a standard breeding method of switchgrass to exploit additive genetic variation by increasing the allele frequency of

desirable traits in synthetic cultivars (Bouton, 2007). There are many successful breeding efforts in switchgrass (Casler, 2012) (Table 1.1).

Table 1.1. Example of improved switchgrass via breeding (Casler, 2012).

| Cultivars | Ecotype | Ploidy | Year of release | Traits selected | USDA hardiness zones |
|---|---|---|---|---|---|
| EG2101 | Upland | 8x | 2009 | Biomass yield, spring vigor, rust resistance | 4, 5, 6 |
| Pathfinder | Upland | 8x | 1967 | Biomass yield and vigor | 4, 5 |
| Shawnee | Upland | 8x | 1996 | IVDMD, biomass yield | 5, 6, |
| Sunburst | Upland | 8x | 1998 | Large seed size and mass | 3, 4, 5 |
| Trailblazer | Upland | 8x | 1984 | IVDMD, biomass yield | 4, 5 |
| Summer | Upland | 4x | 1963 | Earliness, rust resistance | 4, 5 |
| BoMaster | Lowland | 4x | 2006 | IVDMD, biomass yield | 6, 7, 8 |
| Colony | Lowland | 4x | 2009 | Biomass yield, spring vigor, rust resistance | 8, 9, 10 |
| EG1101 | Lowland | 4x | 2009 | Biomass yield, spring vigor, rust resistance | 8, 9, 10 |
| Performer | Lowland | 4x | 2006 | IBDMD, biomass yield | 6, 7, 8 |
| TEM-LoDorm | Lowland | 4x | 2007 | Reduced post-harvest seed dormancy | 6, 7, 8 |

Genetic resources of switchgrass are from some commercial cultivars propagated by crown separation prominent in landscape ornamentation such as ‘Cloud Nine’, ‘North Wind’, ‘Dallas Blues’, ‘Heavy Metal’, ‘Shenandoah’, ‘Rotstrahlbush’, and ‘Warrior’. However, the majority of switchgrass genetic resources is from natural collections of source-identified remnant prairies for agriculture and conservation purposes (Casler, 2012). In addition to the recent domestication, this natural collection without directional selection supports that there is huge genetic diversity within the populations. Table 1.2 lists some minimal or no selection in switchgrass germplasms, such as lowland ‘Kanlow’ from Northern Oklahoma, upland ‘Cave-in-Rock’ from Southern Illinois, and upland ‘Shelter’ from Central West Virginia.

Table 1.2 Examples of switchgrass with no trait-specific selection (Casler, 2012).

| Cultivars | Ecotype | Ploidy | Year of release | Geographic origin | USDA hardiness zones |
|---|---|---|---|---|---|
| Alamo | Lowland | 4x | 1978 | Southern Texas | 6, 7, 8, 9 |
| Kanlow | Lowland | 4x | 1963 | Northern Oklahoma | 6, 7 |
| Dacotah | Upland | 4x | 1989 | Southern North Dakota | 2,3, 4 |
| High Tide | Upland | 4x | 2007 | Northeastern New Mexico | 4, 5, 6 |
| Blackwell | Upland | 8x | 1944 | Northern Oklahoma | 5, 6, 7 |
| Carthage | Upland | 8x | 2006 | North Carolina | 5, 6, 7 |
| Cave-in-Rock | Upland | 8x | 1973 | Southern Illinois | 4, 5, 6, 7 |
| Shelter | Upland | 8x | 1986 | Central West Virginia | 4, 5, 6 |

To produce switchgrass, the target area for production is marginal land to reduce food-fuel competition. An example of such marginal land is mined land reclamation sites with a pH of 3.7 with metal contaminants. Switchgrass can tolerate a wide pH range of 5 to 8 for seed germination (Hanson and Johnson, 2005). Its high adaptability and tolerance enables it to grow on the marginal land (Stucky et al., 1980). Land preparation is crucial for perennial production. The most required fertilizers are a combinations of N plus lime for some pH adjustment that can improve yields by 5 to 30% (Jung et al., 1988). In contrast, most of the studies of fertilization of P and K showed no or little response of yield (Hall et al., 1982; Morris et al., 1982; Panciera and Jung, 1984; Jung et al., 1988; Brejda, 2000). Interestingly, Bentivenga and Hetrick (1991) suggested the link between P and soil microbes. The application of the fungicide benomyl (benzimidazole) to soils of native prairies reduced the growth, but not if P fertilizer was applied.

The field can be either tilled or not tilled. Tillage can disrupt soil borne plant pathogen life cycles, but the tilled field needs to be packed before seeding. No tillage

provides advantages of soil moisture, minimum soil erosion, and less financial input (Parrish and Fike, 2005). The main propagation of switchgrass is from seeds. Prior to seeding, the simplest way to break seed dormancy is to keep the seeds at 23$^{o}$C for 180 days (Zarnstorff et al., 1994). The ideal depth is 1.5 cm (Zhang and Maun, 1990). The recommended seed rating is 4 to 10 kg·ha$^{-1}$ (Moser and Vogel, 1995; Wolf and Fiske, 1995; Vogel, 2000; Barnhart and Miller, 2003). Seeding was suggested in early-April in the Southern U.S. states, which varied depending on locations, and the establishment normally takes three years (Evers and Parsons, 2003; McLaughlin and Kszos, 2005b).

Annual harvest for biomass purpose was suggested near anthesis, or late August depending on the locations to lower nitrogen content in the tissue (Vogel, 2000). Although as mentioned above the five-year production of Kanlow, Alamo, and Cave-in-Rock in the upper southeastern U.S. provided consistent yields (Fike et al., 2006), another long-term study revealed that the yield of Cave-in-Rock suffered from smut caused by *Tilletia maclaganii* (Berk.) Clint. (Gravert et al., 2000). This research has raised concerns of diseases threatening switchgrass production.

Among research related to switchgrass, only 28 of the 1693 research articles directly related to diseases in switchgrass based on AGRICultural OnLine Access (AGRICOLA) (2018) (Figure 1.4). To manage diseases in switchgrass, more studies on the topic need to be more emphasized. In addition to switchgrass smut, rust caused by *Puccinia novopanici* Schw., sharp eyespot caused by *Rhizoctonia cerealis* E.P. van der Hoeven, anthracnose caused by *Colletotrichum navitas* J.A. Crouch, and Bipolaris seed rot and Bipolaris leaf spot caused by *Bipolaris oryzae* (Breda de Haan)

Shoemaker have been reported as deleterious fungal diseases in switchgrass (Cornelius and Johnston, 1941; Zeiders, 1984; Etheridge et al., 2001). Zeiders (1984) emphasized that "if switchgrass is grown to any great extent in the humid areas of the eastern and northeastern USA, [Bipolaris leaf spot] will probably be the most important disease." The speculation was later confirmed with many reports of Bipolaris leaf spot (Krupinsky et al., 2004; Tomaso-Peterson and Balbalian, 2010; Waxman and Bergstrom, 2011; Vu et al., 2013).

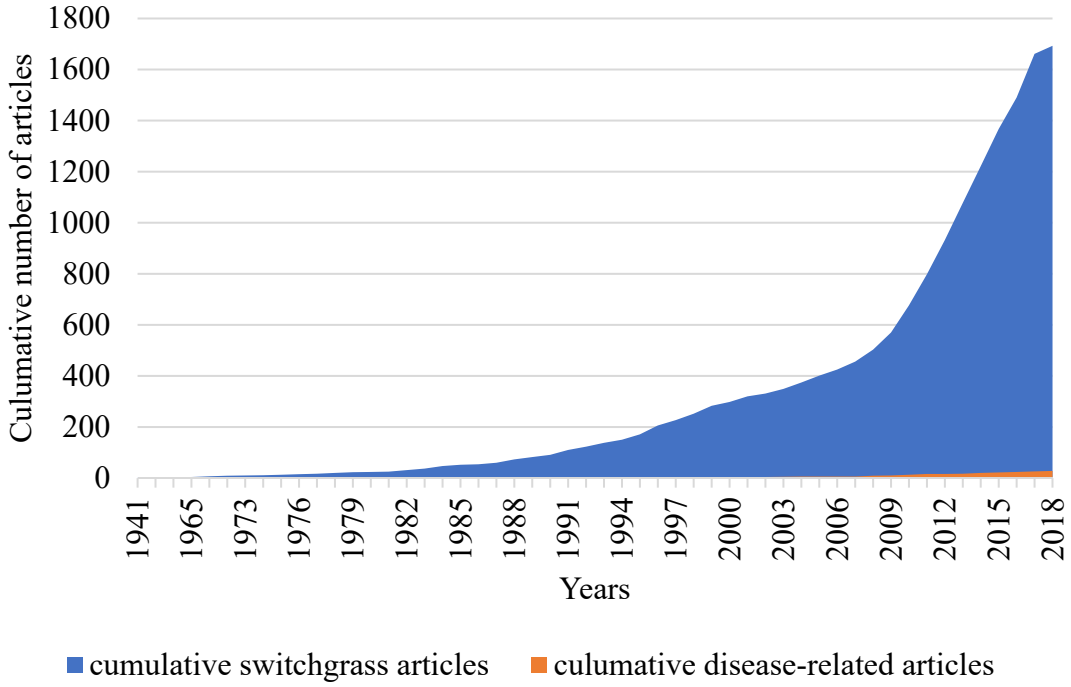


Figure 1.4. Cumulative number of articles of switchgrass in general (blue) and disease-related (orange) from 1941 to 2018 in the AGRICOLA database (2018).

**Plant Immune System**

To manage diseases in switchgrass, understanding the overall plant defense mechanism is important. The mechanisms against pathogens are complicated in that they can be expressed constitutively or induced by the pathogen attack (Thordal-Christensen, 2003; Glazebrook, 2005; Boyd et al., 2013). The most recognized model explaining the defense mechanism is the 'zigzag' model (Figure 1.5) (Jones and Dangl, 2006). To begin with the basal defense, pathogen (microbe)-associated molecular patterns (PAMPs), which are conserved molecules within pathogen species, are recognized by plant pattern recognition receptors (PRRs). The recognition then activates the basal resistance or PAMP-triggered immunity (PTI). In some pathosystem, PTI is sufficient to be resistant to the pathogen invasion (Tsuda and Katagiri, 2010). Successful pathogens overcome PTI by secreting virulent effector proteins or host-specific toxins. Such effectors cannot only suppress PTI but also adjust the host metabolism to be beneficial to the pathogen growth and reproduction (Friesen et al., 2008; Vleeshouwers and Oliver, 2015). These effectors induce the second line of defense called effector-triggered immunity (ETI) by the plant receptors encoded from specific disease resistance (*R*) genes. In a biotrophic pathogen invasion, gene-for-gene interaction with *R*-genes provides a hypersensitive response that leads to localized programmed cell death, stopping further invasion. The *R*-genes generally encode the nucleotide binding site-leucine-rich repeat (NB-LRR) class of proteins that effectively specify the pathogen effector (Hammond-Kosack and Jones, 1997). However, *R*-genes are not always durable because the pathogen can quickly mutate the effectors not to be recognized by the plant's *R*-genes. Plant breeding, therefore,

focuses more on quantitative resistance by stacking many genes with small effects to provide durability (Johnson, 1984).

However, such gene-for-gene interaction conferring resistance by *R*-genes cannot be applied to necrotrophic pathogen interaction with the host (Friesen et al., 2008). The necrotrophic pathogen secrets host-specific toxins to interact with host sensitivity genes, resulting in a compatible susceptible interaction that creates a suitable condition for the pathogen. This interaction is an inverse gene-for-gene model called effector-triggered susceptibility (ETS) (Friesen et al., 2007). In breeding for resistance to such pathosystem, the susceptible individual carrying the necrotrophic effectors can be excluded out of the population.

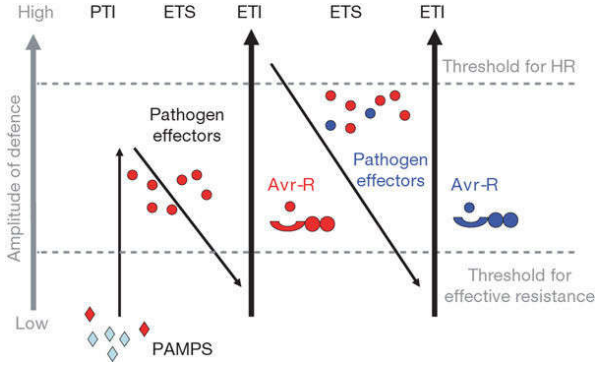


Figure 1.5. 'Zigzag' model of host-pathogen interaction. The base line immunity detects microbial/pathogen-associated molecular patterns (MAMPs/PAMPs, red diamonds) via PRRs to trigger PAMP-triggered immunity (PTI). If the pathogen can overcome PTI, it delivers effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS). Later, one effector (indicated in red) is recognized by an NB-LRR protein, activating effector-triggered immunity (ETI), an amplified version of PTI that often passes a threshold for induction of hypersensitive cell death (HR). Lastly, the pathogen can mutate to lose the red effector or gain new effectors through horizontal gene flow (in blue)—these can help pathogens to suppress ETI. Selection can introduce new NB-LRR alleles that can recognize the new pathogen's effectors, resulting again in ETI (Jones and Dangl, 2006).

## Bipolaris oryzae

### Taxonomy, characteristics and life cycle

The ascomycete fungus *Bipolaris oryzae* (Breda de Haan) Shoemaker (teleomorph: *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur) is one of the most prevalent switchgrass pathogens that has been reported in New York, Tennessee, Mississippi, and North Dakota (Krupinsky et al., 2004; Tomaso-Peterson and Balbalian, 2010; Waxman and Bergstrom, 2011; Vu et al., 2013). As the name suggested, the fungus is one of the most devastating rice pathogens (Ou, 1985). Most studies have been conducted in rice (*Oryza sativa* L.). Thus, the *B. oryzae* – rice interaction is used as a model for the *B. oryzae* – switchgrass interaction. *Bipolaris oryzae* is a necrotrophic fungus that induces and utilizes cell death. In a broader infection mechanism, *B. oryzae* can survive in soil for many years in the absence of host debris (Ou, 1985) and is also seed-borne (Damicone et al., 1992). In temperate areas, *B. oryzae* can survive in seeds, as the seeds provide the primary inoculation, whereas the secondary inoculation is from the wind-borne asexual conidia Figure 1.6) (Ou, 1985; Barnwal et al., 2013). In rice, the infected seeds confirmed that the Bipolaris mycelia reside in all parts of the seed including embryo, endosperm, palea, lemma, rachilla, and sterile lemma (Van Ba and Sangchote, 2006). The dormant mycelium in these tissues is reactivated during germination and cause lesions on roots and coleoptiles (Nyval, 1989; Nghiep and Gaur, 2004). The same symptoms also occur in switchgrass seeds (Figure 1.7). Moreover, the transmission rate from seed to seedling can be as high as 80% (Toledo et al., 2006). In this study, the reduction in

seed germination, the infection of coleoptile and root after germination, and seedling dying off within three to four weeks are collectively called Bipolaris seed rot.

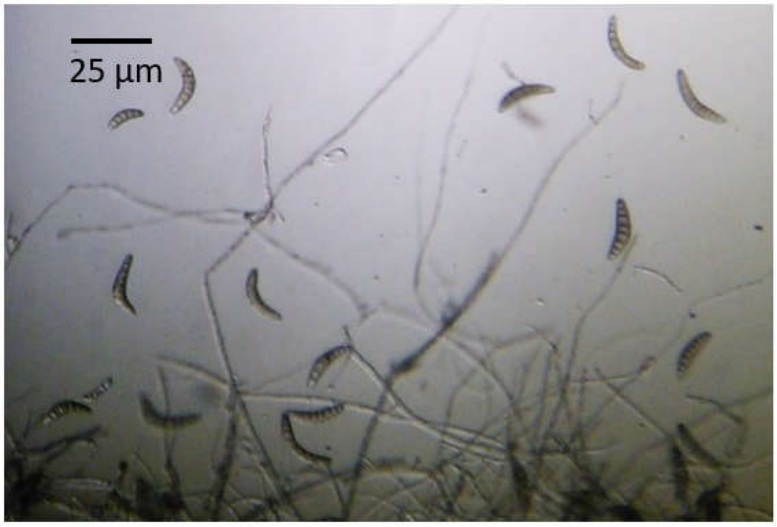


Figure 1.6. Bipolaris conidia and hyphae under a light compound microscope.

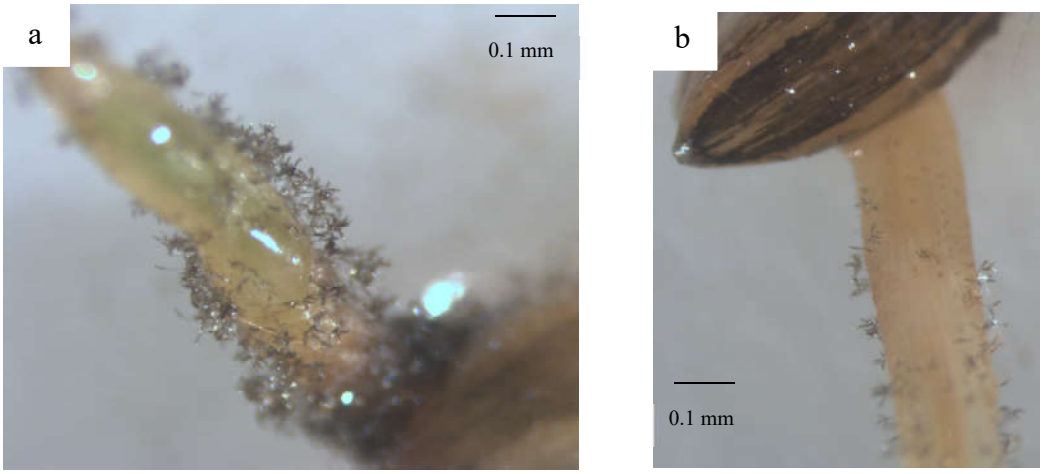


Figure 1.7. Switchgrass seed infestation with Bipolaris oryzae. a) mycelium and conidia form on coleoptile b) infestation on root on germinating seed.

In the infection mechanism for Bipolaris leaf spot, as the name Bipolaris implies, conidia germinate from both ends and form penetration structures called stromatae (Bockhaven, 2014) . After the pathogen penetrates a leaf, the mycelia enter the apoplast. Then they penetrate to the leaf cells and induce host cell death, from which *B. oryzae* can utilize the nutrients. The infected leaf shows the brown lesions in a chlorotic elongated circle (Figure 1.8). In the favorable environment at high humidity, a new generation of conidia is produced in the brown lesions and dispersed aerially. *Bipolaris oryzae* has a short incubation period for conidia (less than 24 hours). The disease can develop within 3-4 days, and the pathogen sporulates approximately 6 days after infection, providing the secondary inoculation (Figure 1.9) (Ou, 1985; Barnwal et al., 2013).


Figure 1.8. Bipolaris lesion on a mature leaf.

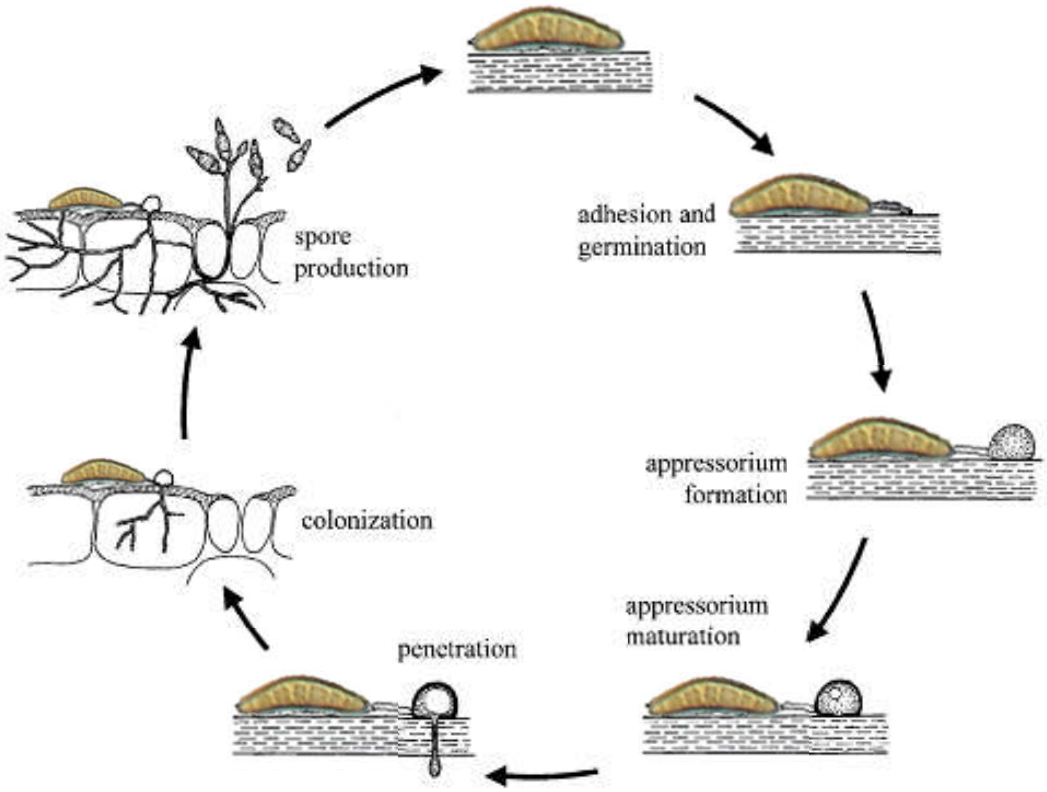


Figure 1.9. Lifecycle of Bipolaris oryzae, adapted from Thines et al. (2004).

Genome comparisons among *Bipolaris spp.* revealed that there are no host-specific toxins produced by *B. oryzae* (Condon et al., 2013). Instead, *B. oryzae* is known for producing two non-host-specific toxins, ophiobolin A and B (Nakamura and Ishibashi, 1958). These two sesterterpenoid phytotoxins inhibit K+ uptake and H+ extrusion, causing plasma membrane depolarization and disorganization due to the capacity of calmodulin inhibition. The plasma membrane disruption is responsible for impairing photosynthesis, respiration, and protein and DNA synthesis. Thus, these phytotoxins induce the cell death of the plant cell, causing lesions on leaves in the ETS system (Chattopadhyay and Samaddar, 1976; Xiao, 1991).

As emphasized above, the long duration of high humidity of the eastern and northeastern USA is favorable for *B. oryzae* (Zeiders, 1984); therefore, New York is expected to have high disease pressure (Waxman and Bergstrom, 2011). Moreover, the target environment for growing switchgrass on marginal land with low input is expected to intensify Bipolaris diseases because nutrient deficient soil conditions showed higher Bipolaris leaf spot in rice (Katara et al., 2010). Additionally, Bipolaris diseases, which reduced grain yield and quality, provided the major contribution to the Bengal famine of 1943 when soil maintenance was scarce (Padmanabhan, 1973).

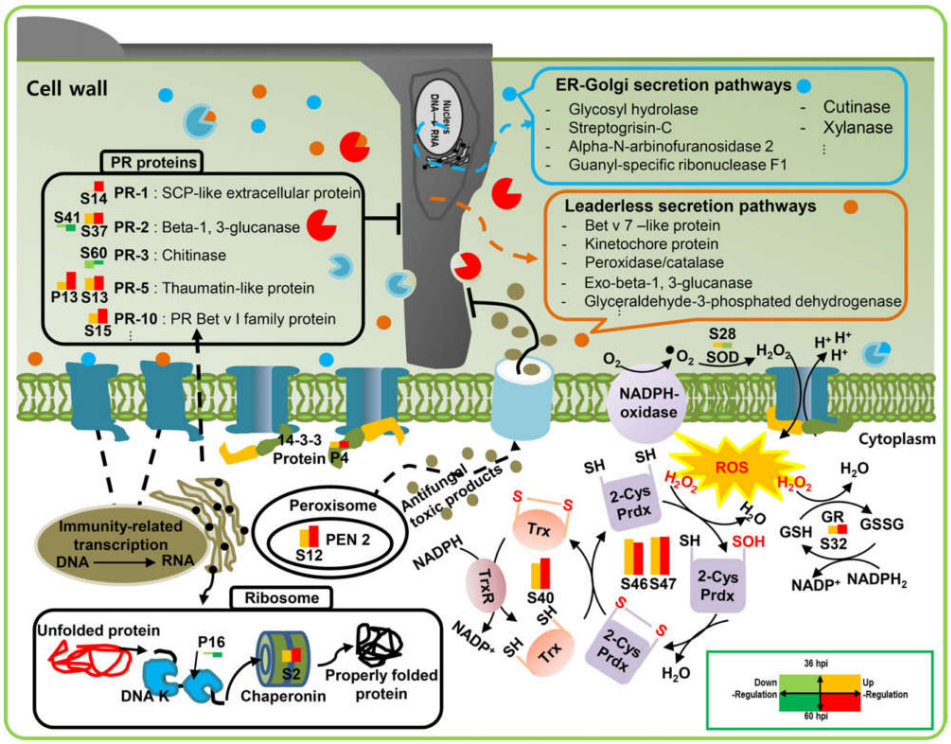


Figure 1.10. Overview of proteomic interaction between host and *B. oryzae*. The response is complicated and mainly involves the detoxification of ROS including SOD, superoxide dismutase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; 2-Cys Prdx, 2-Cys peroxiredoxin; Trx, thioredoxin; and TrxR, thioredoxin reductase (Kim et al., 2014).

**Molecular aspects of switchgrass-Bipolaris oryzae interaction**

The cumulative proteomic responses of rice to *B. oryzae* in both intra- and extra-cellular spaces have been studied (Kim et al., 2014) (Figure 1.10). At the beginning of the invasion, the penetration structure is recognized as PAMP by PRR. In this case, the component of the fungal cell wall is chitin (a polymer of N-acetyl-D-glucosamine) and can be recognized by LysM domain-containing receptor-like kinase1 (LysM RLK1)/chitin-elicitor receptor kinase 1 (CERK1) that relays signals via mitogen-activated protein kinases (MAPKs) (Wan et al., 2008). The host, consequently, secrets chitinase and beta-1,3-glucanase to confer PTI. To overcome PTI, *B. oryzae* secrets Alp1, a protease enzyme. In the same time of penetration, the fungus secrets cell wall degradation enzymes to extracellular spaces via both ER-Golgi and leaderless secretion pathways including $\alpha$-galactosidase, $\alpha$-amylase, endo-1,4-beta-xylanase I, exo-beta 1,3 glucanase, $\alpha$-N-arbinofuranosidase-2, and cutinase precursor. These enzymes break the cell wall and release fungal toxins of ophiobolin A and B to induce programmed cell death showing ETS (Nakamura and Ishibashi, 1958). The host also produces cysteine proteinase inhibitor and polygalacturonase inhibitor to degrade the fungal cell wall degradation enzymes. The induced programmed cell death results in the high amount of reactive oxygen species (ROS) accumulation in damaged plant cells.

The host responds by producing ROS-detoxifying enzymes such as ascorbate peroxidase, superoxide dismutase, and dehydroascorbate peroxidase. Moreover, Calvin cycle is a key contributor for generating energy with the upregulation of

fructose bisphosphate aldolase, sedoheptulose-1,7-bisphosphates, and RuBisCo. In tricarboxylic cycle, oxaloacetate and aspartate aminotransferase are upregulated to produce other amino acids for a defense mechanism. However, homocysteine S-methyltransferase involving ethylene (ET) biosynthesis is found upregulated. The ET-plant immunity is a complicated system. Bockhaven (2014) has proved that the fungus produced ET to trigger the rice host to synthesize ET, which compromised the resistance via senescence extension and inhibition of phenylpropanoid-driven defenses.

**Disease management**

Since switchgrass is not an annual plant, conventional practices such as tillage, crop rotation and delayed seeding, which can interrupt the disease cycle, cannot be adopted for long-term management (Crouch et al., 2009). The establishment year is the most important period to control the disease by soil fumigation with a fungicide such as Bavistin, Hinosan, Tilt 250 EC (Propiconazole), and Dithane M-45 (Ahmed et al., 2002) or by seed coating with fungicides that can increase seedling emergence (Luo et al., 2014). However, fungicide reapplication is not economically feasible since switchgrass has been designed for use on marginal land with as low input as possible. Because of a lack of fungicide reapplication and aerial dispersal of conidia, leaf spot disease can be introduced to fields. Therefore, switchgrass breeding for *B. oryzae* resistance was recommended for sustainable management (Waxman and Bergstrom, 2011; Barnwal et al., 2013).

Breeding for resistance to Bipolaris diseases in switchgrass has never done before, but disease severity in Blackwell, Cave-in-Rock, Dacotah, and Summer inoculated with *B. oryzae* showed variation among populations that can be utilized in selection (Fajolu, 2012). Although the variation of resistance to Bipolaris diseases within a population has never been reported, the restricted outcrossing, high self-incompatibility and recent domestication suggested high genetic variance within a population. Heritability of the resistance to Bipolaris diseases has also never been estimated. In comparison to switchgrass rust, the broad-sense heritability of the resistance to rust of upland ecotypes ranged from 0.51 to 0.96, whereas the broad-sense heritability of lowland ecotypes ranged from 0.0 to 0.78 (Eberhart and Newell, 1959). This wide range of heritability estimates is expected for resistance to Bipolaris diseases in switchgrass, suggesting the possibility for recurrent phenotypic selection or genomics-assisted breeding. Since *B. oryzae* is a necrotrophic pathogen, the main goal for breeding against the pathogen is to exclude individuals inheriting susceptible alleles and possibly increase the frequency of resistance alleles in the population.

Although there were no confirmed resistance genes against *B. oryzae*, three quantitative trait loci (QTLs) have been identified in rice on chromosomes 1, 4, and 11 (Sato et al., 2008, 2015; Katara et al., 2010). These QTLs are useful to compare with resistance in switchgrass. Most of the switchgrass breeding programs are based on regional adaptation (Table 1.1). A spaced planting in a switchgrass nursery consisting of 1,000 to 10,000 plants can be used to successfully improve yield and IVDMD (Casler, 2012). In breeding for disease resistance, the disease pressure needed to be controlled and intensified; therefore, the screening of seedlings in a greenhouse was

performed in many crops (Knudsen et al., 1995; Nghiep and Gaur, 2004; Juliana et al., 2018). The selection in greenhouse conditions, however, need to consider the transferability of resistance from greenhouse to a field (Foolad et al., 2000; Twizeyimana et al., 2007).

Alternatively, although fungicide application is not suitable for switchgrass due to the anticipated low investment and the perennial cycle, an effective chemical that could be applied in the establishment year and last until the end of production period would be an ideal candidate. According to Bockhaven (2014), *B. oryzae* resistance in rice can be promoted by silicon application. He found that silicon could not only prevent physically the growth and penetration of *B. oryzae* through the epidermis of a rice leave but also triggered the defensive signal from root to shoot to accumulate silicon. Silicon also prevented *B. oryzae* from taking over the host ethylene synthesis pathway. Moreover, since residual silicon can persist in the soil over time, annual reapplication is not required (Alvarez and Datnoff, 2001). It is worthwhile regarding feasibility and investment benefit to include research on silicon application for disease management of switchgrass. Therefore, silicon application can be a good candidate for leaf spot control. However, such a study has not been done yet in switchgrass.

Despite this potential, it is important to note that higher silicon accumulation in feedstock can cause severe problems during high-temperature (approximately 700°C) conversion and combustion processes in power plants from oxidized slag and corrosive alkali sulfates which impede industrial investment (Miles et al., 1996). Thus,

silicon accumulation trade-off should be prudently considered between preventing biomass loss from the disease and reducing conversion capacity.

To confer the resistance by silicon, the silicon transportation and accumulation are the key features of three transporter proteins including Lsi1 and Lsi2 in roots and Lsi6 in shoots (Figure 1.11) (Rodrigues and Datnoff, 2015). In rice, Lsi1 membrane protein at the distal side of exo- and endodermis cells is similar to the water channel proteins (aquaporins) that take up silicon in the form of silicic acid ($H_2SiO_4$) via the passive mechanism (Jian et al., 2006; Ma et al., 2007; Moore et al., 2011). In the same cell but at the proximal side, Lsi2 transporters pump the silicic acid from exodermis cells to parenchyma via the active mechanism. Later, endodermal cells take up the silicic acid from arenchyma via Lsi1 passively and pump the acid to cortical cells via Lsi2. The silicic acid is transported to xylem via an unknown protein. The leaf takes up silicic acid via Lsi6 passively. In the leaf, water-soluble silicic acid polymerizes into insoluble silica ($SiO_2.nH_2O$,). The polymerized silica can be both inside the cell as phytoliths and colloidal cytoplasmic silica, and outside the cell as a silica layer or silica bodies located just beneath the cuticle (Yamanaka et al., 2009; Moore et al., 2011). Although the actual silicon translocation has never been studied in switchgrass, a similar mechanism was expected because the gene-expression by qRT-PCR revealed the existence of silicon transporter, Lsi1 and Lsi2 in tetraploid switchgrass (Palmer et al., 2014).

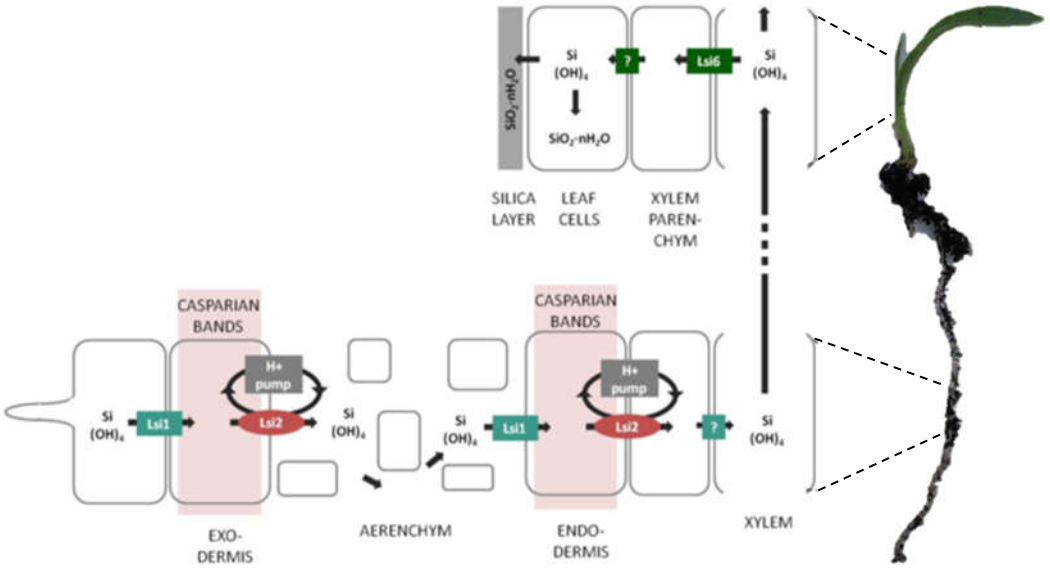


Figure 1.11. Expected overall silicon uptake and accumulation from root to shoot of switchgrass, adapted from (Moore et al., 2011).

## Conclusion

Genetic diversity appears to be a viable method in switchgrass to improve the populations via both recurrent phenotypic selection and genomics-assisted selection. *Bipolaris oryzae* was identified as one of the most devastating pathogenic fungi causing Bipolaris seed rot and leaf spot. To maintain high biomass yield of switchgrass, disease management is crucial. Breeding for resistance as well as silicon amendment are both potential approaches to reduce Bipolaris infection. This expectation was because the high heritability of resistance to rust could infer the possibility of such heritability of resistance to Bipolaris diseases, and silicon amendment was proven to confer resistance to BLS in rice. Therefore, the objectives of this research were to 1) establish a screening technique for selection for resistance to BSR and BLS, 2) estimate heritability of the resistance to these diseases, 3) conduct two cycles of recurrent phenotypic selection for the resistance, 4) provide potential markers for genomics-assisted selection via GWAS associated with BLS, and 5) study the effects of silicon on resistance to BSR and BLS in switchgrass.

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# CHAPTER 2

## ASSESSMENT OF DISEASE EVALUATION TECHNIQUES


### Abstract

*Bipolaris oryzae* (Breda de Haan) Shoemaker is a soil- and seed-borne ascomycete causing two distinct diseases – Bipolaris seed rot (BSR) and Bipolaris leaf spot (BLS) – in a biomass-producing switchgrass (*Panicum virgatum* L.). Breeding for resistance is a potential approach to manage the disease. However, the disease evaluation for screening has never been established. In this study, the validation of inoculation for BSR was done in soil and seed inoculations whereas the validation of inoculation for BLS was done in seedlings. The establishment of both types of inoculation was by varying the inoculation conditions. The improvement of heritability estimates was conducted in KL and CIR half-sib progenies. The transferability of resistance to BLS between seedlings in a greenhouse to mature plants in a field was estimated. Also, the non-destructive BLS evaluation in mature plants was determined in detached leaf section and leaf disk assays. As a result, we established the screening method for both BSR and BLS with some cautions of repeatability. The consistency of severity between BLS in seedling and field evaluation was low. The adjustment in tray arrangement and inoculation technique did not improve the heritability of resistance to BLS significantly. The non-destructive approaches showed potential to evaluate BLS in mature plants.

## Introduction

Switchgrass (*Panicum virgatum* L.) is a North America native perennial grass and considered as one of the most reliable biofuel crops because of its high biomass with low input requirement, well-adapted to marginal and poor land, and high energy capacity (Sanderson et al., 2006; Schmer et al., 2008; Lee et al., 2009). Besides the improvement of biomass, yield maintenance is also important. Diseases have been reported to reduce biomass in switchgrass (Thomsen, P. M., Brummer, E. C., Shriver, J. M., and Munkvold, 2008; Crouch et al., 2009; Fajolu et al., 2012; Uppalapati et al., 2013). *Bipolaris oryzae* (Breda de Haan) Shoemaker (BO) is one of the reported fungi reducing yield and field establishment in switchgrass (Fajolu et al., 2012).

Bipolaris seed rot (BSR) and Bipolaris leaf spot (BLS) are two major diseases from BO. The effect of BSR can be noticed from the reduction in germination. The hyphae and spore (conidia) of BO can be found on the seed surface (Figure 1.7a and b). The fungus can also infest the germinating shoot and root (Figure 1.7c and d). This post germination symptom resulted in dead seedlings in week 3 to 4 after planting (Fajolu, 2012). In its life cycle, BO is a soil- and seed-borne pathogen that can live saprophytically in soil and crop residue, and on the seed surface before transferring to seedlings or mature plants to cause BLS. The high BLS pressure can reduce biomass yield up to 50%.

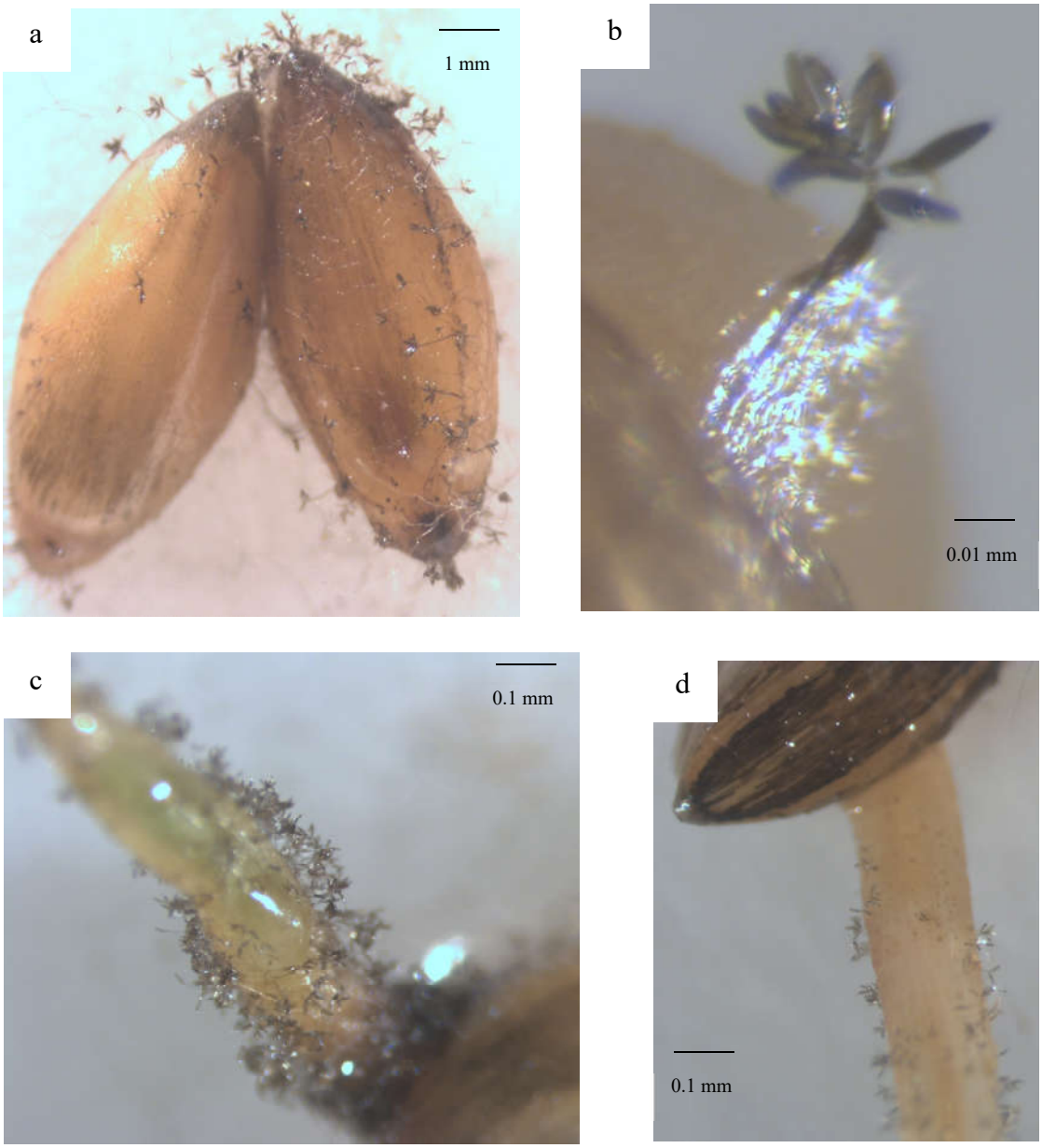



Figure 2.1. Switchgrass seed infestation with Bipolaris oryzae (BO). a) the seeds covered with hyphae and conidia of BO; b) BO conidia; c) BO infestation on shoot; d) BO infestation on root on germinating seed.

To manage the disease, breeding is one of the most economical approaches. Before selection, the phenotyping methods for evaluating BSR and BLS needed to be established. The inoculations in seed for BSR was conducted in rice (*Oryza sativa* L.*)* (Van Ba and Sangchote, 2006). Fajolu (2012) has inoculated both seed and soil to test

BSR in switchgrass, but it was tested in pots filled with 100 seeds. For selection, adjustments need to be made in: inoculation duration, concentration for high enough disease pressure to make selection progress, and determining optimal time to record BSR. The inoculation for BLS has been done in various stages of rice from seedlings to mature rice (Sato et al., 2008, 2015). Fajolu (2012) also conducted BLS evaluations in seedlings and mature switchgrass. However, the method still needed adjustment for screening larger populations in selection by determining reliable scores, varying inoculation condition, and improving inoculation technique. Additionally, the non-destructive BLS evaluation is important to assess the BLS in the mature plant by reducing GxE effects and increasing disease pressure under control conditions. The detached leaf and leaf assays are common approaches for this purpose (Moriwaki et al., 2006; Lee et al., 2007; Fajolu, 2012). Similar to other approaches, the detached leaf and leaf disk assay need validation by estimating repeatability.

The objectives of this study were to 1) determine effective inoculation techniques and conditions for screening for resistance to BSR in seeds, and resistance to BLS in seedlings, 2) estimate the correlation between resistance to BSR and BLS in unselected populations to prepare for a breeding program, and 3) determine the non-destructive disease assessment for resistance to BLS in mature plants.

## Materials and Methods

### Bipolaris isolate source and inoculum preparation

In this research, *B. oryzae* isolate Bo008NY07 was the only single-spore isolate utilized. The spore was isolated from a 'Carthage' switchgrass leaf in the warm season biofuels field experiment in Ithaca, NY, by Katie Waxman in 2007 (Waxman

and Bergstrom, 2011). The spore isolate of *Bipolaris oryzae* was subcultured on one-strength potato dextrose agar (PDA). On the inoculation day, three-week-old *B. oryzae* in Petri dishes were flooded with sterile deionized water and scraped with a glass spreader to dislodge and obtain conidia. The residue of PDA and mycelium in the conidia suspension was removed via gauze. The concentration of conidia was then adjusted by hemocytometer under a microscope. Two drops of Tween-20 were added to 100 mL of inoculum as a surfactant.

**Plant material**

In this study, there were four sources of plant materials including bulked seeds from seed stocks as a based population, half-sib seeds from 'Cave-in-Rock' (CIR) as an upland representative and 'Kanlow' (KL) as an lowland representative, and mature plants from selected half-sibs of CIR and ST (Chapter 3), and mature plants from the Northern association panel as a broader population. The seeds of 'Cave-in-Rock' (SWG 07-338), 'Shawnee' (Panvir 05-09), and 'Shelter' (SWG 07-310) were from the collection of Ernst Conservation Seeds, Inc. 'Kanlow' (NY12-301) and 'Blackwell' (NY12-302) were from bulked half-sib seed in production nursery in Ithaca, NY. All of the seeds were surface-sterilized by 95% ethanol for 1 minute, 10% sodium hypochlorite for 1 minute and three times of deionized water for 1 minute.

**Half-sib seed production**

'Cave-in-Rock' and 'Kanlow' seedlings were planted in 20 × 10-cell trays in a greenhouse at 30°C with 12-hour photoperiod of supplemental light by high-pressure sodium lamps at 40 klm and ambient temperatures of 18 to 29°C in February 2014. Both cultivars were transplanted in 10-cm plastic pots in the greenhouse in April 2014.

One-hundred-twenty-six CIR seedlings were selected and acclimatized in cold frames in April 2014 and then grown in a space-transplanted nursery with 0.9 m apart from each plant in Ithaca, NY. Due to being an upland ecotype, ‘Cave-in-Rock’ started flowering in July, was allowed random wind pollination, and hand-harvested at the beginning of October. In contrast, ‘Kanlow’ seedlings continued being transplanted in 30-cm clay pots in the same greenhouse to avoid winter damage since it started flowering later than ‘Cave-in-Rock’ in September. During the peak of anthesis in November, 114 pots of ‘Kanlow’ were randomly moved every two days to reduce the bias from the location. The plants were shaken every day at 10:00 am to stimulate pollination. The ‘Kanlow’ seeds were hand-harvested at the end of December. The seeds were processed to remove glumes and lemmas by rubbing the seeds in a rubber tube. Seeds were surface-sterilized with 95% ethanol for 1 minute, 10% sodium hypochlorite for 1 minute and three times of deionized water for 1 minute. After drying, seeds were stored by each half-sib at room temperature.

**Mature plants in the Northern Association Panel**

Some of the mature plants of the Northern Association Panel were used to determine the non-destructive evaluation approach. This population was developed for accelerated breeding progress especially for bioenergy traits at northern latitude (Lu et al., 2013; Lipka et al., 2014). It consists of 479 genotypes from 66 populations representing mostly upland northern populations and some southern lowland populations. They were first planted in Ithaca, NY, in 2008 and then vegetatively cloned and planted in a randomized, complete block design with three replicates in Ithaca, NY, and Philipsburg, PA, in 2016 without any fungicide applications.

**Determine seed inoculation technique for BSR**

***Soil inoculation***

Since BO is also a saprophytic fungus, soil inoculation was examined to determine the effectiveness for screening for BSR. The soil inoculation was adjusted from Fajolu (2012). The 25 seeds of CIR, 'Blackwell' (BW), KL, and 'Shawnee' (SN) were planted in three 100-cell trays filled with Cornell Peat-lite media. After the seeds were covered with the media, two mL of inoculum of BO conidia at 0, $5x10^3$, or $10^4$ conidia·$mL^{-1}$ were applied to each cell. The trays were kept in a greenhouse with 7-hour light and watered once daily. The number of germinating seeds were collected at three weeks after inoculation.

***Seed inoculation***

In addition to being a saprophyte, BO is a seed-borne pathogen (Ou, 1985). The seed inoculation was modified from Fajolu (2012). First, in my research the inoculation duration was verified. The 0.2 g seeds of BW and KL were soaked in 25 mL BO inoculum of 0, $10^3$, and $10^5$ conidia·$mL^{-1}$ and shaken at 60 rpm for two different durations – 10 minutes and 24 hours. The inoculated seeds were then drained of the inoculum and dried at room temperature overnight. One hundred seeds of each treatment were planted in 100-cell trays filled with Cornell Peat-lite media in greenhouse and watered once daily. The numbers and heights of germinating seeds were collected at three weeks after inoculation.

Second, the suitable date for collecting the germination data was conducted in BW, CIR, KL, SN, 'Sunburst' (SB), and 'Shelter' varying the inoculum concentration from 0 as control, $10^3$, $10^4$, to $10^5$ conidia·$mL^{-1}$. One hundred seeds from each

treatment and cultivar were planted in 100-cell trays, kept in the greenhouse and watered once every day. The number of germinating seeds were collected at 1, 2, 3, and 4 weeks after inoculation.

Third, the suitable concentration of inoculum for seed inoculation was determined in BW, CIR, KL, and ST in a randomized, complete block design (RCBD) with 18 replicates in 288-cell trays. The seeds were inoculated in the ratio of seeds: inoculum at 0.2 g seeds:25 mL inoculum. The inoculum concentrations were varied from 0 as control, $10^3$, $10^4$, to $10^5$ conidia·mL$^{-1}$. The number of germinating seeds were determined in week 4. The reduction percentages of germination were computed as

$$\%germination\ reduction = \frac{\%germination\ of\ inoculated\ seeds - \%germination\ of\ control\ seeds}{\%germination\ of\ control\ seeds}$$

**Determine seedling inoculation technique for BLS in seedlings**

***Determine scale for disease evaluation***

Bipolaris leaf spot is recommended to be evaluated by area under the disease progress curve (AUDPC) (Kumar et al., 2011); however, the percentage of leaf covered by lesions (PLC) was also utilized (Fajolu et al., 2013). In this study, the severity of BLS was evaluated based on PLC from 0 to 100 increasing by ten as in Figure 2.2. The AUDPC and PLC were compared to determine the most reliable evaluation for further selection. The sterile seeds of BW, CIR, KL, and SN were planted in 20 × 10-plot trays for three replications. Each cultivar was randomly planted in five consecutive rows in total of 50 seeds. Four-week-old seedlings were sprayed with a spray bottle with inoculum concentrations at 5 ×$10^3$ and $10^4$

conidia·mL$^{-1}$. The seedlings were sprayed thoroughly until the presence of droplets of inoculum on leaves. Each tray was covered with a clear plastic bag for 24 hours to stimulate conidia germination. The inoculated trays were kept in a greenhouse with a 12-h photoperiod and watered twice daily. The PLC was evaluated on at 1, 4, 7 and ten days post inoculation (dpi). The AUDPCs were calculated based on the standard method from PLCs (Simko and Piepho, 2012)

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where y is the PLC and t is the day of evaluation.

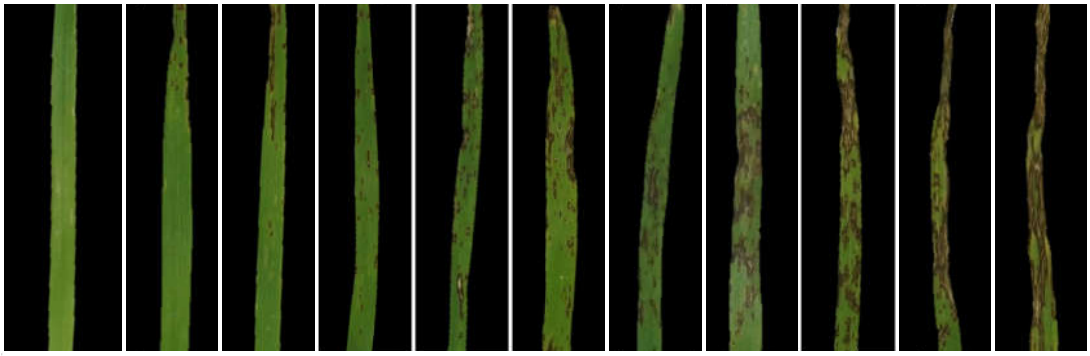



Figure 2.2. Severity scores for BLS in percentage of leaf covered with lesions (PLC) with ten increments from Cave-in-Rock at 7 dpi.

### *Determine seedling inoculation concentration*

After determining the score for rating BLS, the concentration variation was conducted in four cultivars including BW, CIR, KL, and ST to determine the concentration of BLS. Surface-sterilized seeds of the four cultivars were planted in a 288-cell tray filled with Cornell Peat-lite media. More than one seed was planted in one cell to make sure each cell contained one seedling. Extra seedlings were rogued out one week before inoculation. On week 4, three trays were inoculated by the same concentration inoculum. The concentrations included control as 0, $10^3$, $10^4$, and $10^5$ conidia·mL$^{-1}$. The inoculum was applied by a spray bottle until the presence of droplets over seedlings. Each tray was covered with a clear plastic bag to increase humidity for 24 hours. The severity was evaluated at seven dpi as PLC. The Pearson's pairwise correlation was calculated in JMP version 13.

### *Effects of seedling inoculation on dry weight and height*

In addition to adjusting seedling inoculation, the effects on seedling dry weight and height were examined. The 4-week-old seedlings of BW, CIR, KL, and SN in each 200-cell tray were inoculated with 0, $10^4$, and $10^5$ conidia·mL$^{-1}$ in three replicates. The inoculum was applied by a spray bottle until the presence of droplets over seedlings. The severity of BLS and height were evaluated as PLC at seven dpi. The above ground shoot of each seedling was dried at 35ºC for a month and weighted after completely dried. The correlations among PLC, weight, and height were estimated.

### *Correlation between BSR and BLS*

Since BO can cause both BSR and BLS in switchgrass, the correlation between the two traits was determined by comparing the reduction percentage of germination for BSR with PLC of seedlings for BLS with and without seed inoculation. The sterilized seeds of BW, CIR, KL, and SN were inoculated with 0, $10^3$, 5 x $10^3$, $10^4$, and $10^5$ conidia·mL$^{-1}$ inoculums and planted in each different 288-cell tray. Germination was determined on week 4, and the infected seedling was removed before seedling inoculation. The inoculum of 0, $10^3$, 5 x $10^3$, $10^4$, and $10^5$ conidia·mL$^{-1}$ was applied by a spray bottle until the presence of droplets over seedlings. The tray was covered with a clear plastic bag for 24 hours, and the PLC was evaluated at seven dpi. The combinations of different concentrations between seed and seedling inoculation were compiled from eight separate experiments.

### Identify tray-tray variations

Since the selection is recommended by screening large sample sizes, selection needed to be conducted across multiple trays. The tray-tray variation was determined. Blackwell seedlings were planted in ten 200-cell trays filled with Cornell Peat-lite media. The $10^5$ conidia·mL$^{-1}$ inoculum was applied by a spray bottle until the presence of droplets over all seedlings. The trays were covered with clear plastic bags for 24 hours, and the PLC was evaluated at seven dpi.

### Improving inoculation technique for heritability estimates

Since heritability estimate experiments were conducted while the selections for resistance to Bipolaris leaf spot progressed, the experiments were changed in inoculation techniques and plot designs. The first estimation was conducted in seven

200-cell trays per replicate for three replicates. Each tray contained 17 to 18 half-sib progenies (125 progenies in total) with resistant ST and susceptible BW check randomly assigned in two rows (Figure 2.3a). Four-week-old seedlings were sprayed with 40 mL of $10^5$ conidia·mL$^{-1}$ by a spray bottle. The second heritability estimation was similar to the first heritability but discarded low germination half-sibs to 16 or 17 half-sib progenies per tray (115 progenies in total) (Figure 2.3b). Four-week-old seedlings were sprayed with 20 mL of $10^5$ conidia·mL$^{-1}$ by an airbrush at 20 psi. The third heritability estimate (experiments 3, 4, and 5) was conducted with five replicates (Figure 2.3c). An entire replicate was within one 288-cell tray, in which 47 half-sib progenies and only BW were randomly assigned in five replicates. Four-week-old seedlings were sprayed with 20 mL of $10^5$ conidia·mL$^{-1}$ by an airbrush. Each tray was covered with a clear plastic bag to simulate natural infection. After 24 hours of incubation, the plastic bag was removed. Inoculated seedlings were kept in the greenhouse to evaluate PLC at seven dpi.

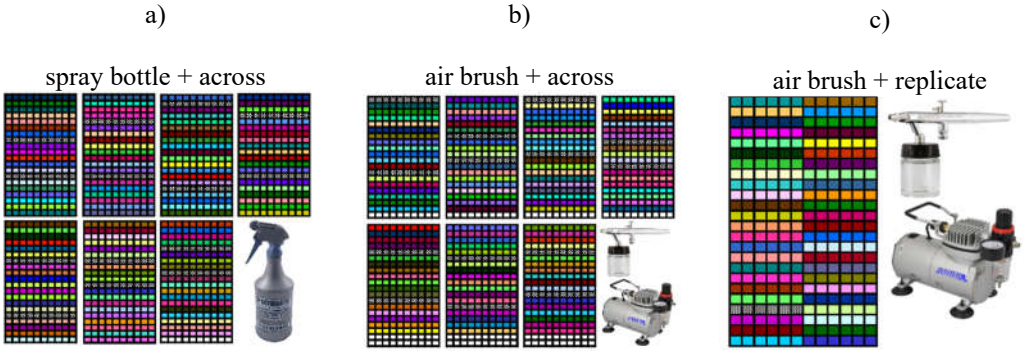



Figure 2.3. Improvement of experimental design for heritability estimates of half-sib progenies of CIR and KL in 12x24-plot trays as a) spray bottle + across multiple trays per replicate, b) air brush + across multiple trays per replicate, and c) air brush + replicate within tray. Each color represents each half-sib progeny.

**Narrow-sense Heritability estimate**

We aimed for estimating the narrow-sense heritability of resistance to BLS based on individual and half-sib selections to determine the effectiveness of selection in 'Kanlow' and 'Cave-in-Rock' as representatives of lowland and upland switchgrass, respectively. Narrow-sense heritability estimation was based on case 1: Analysis on an individual plant basis (Nguyen and Sleper, 1983). Standard error was calculated under the assumption that phenotypic variance is constant (Dickerson, 1969).

For individual heritability:

$$h^2_{individual} = \frac{\sigma^2_A}{\sigma^2_P} = \frac{4\sigma^2_F}{\sigma^2_F + \sigma^2_e + \sigma^2_w}$$

$$s.e.(h^2_{individual}) = \frac{4\sqrt{\frac{2}{(rn)^2}\left(\frac{MSF^2}{df_F + 2} + \frac{MSE^2}{df_e + 2}\right)}}{\sigma^2_F + \sigma^2_e + \sigma^2_w}$$

where $\sigma^2_A$ = additive genetic variance

$\sigma^2_P$ = phenotypic variance

$\sigma^2_F$ = family variance component

$\sigma^2_e$ = error variance component or plot-to-plot environment variance

$\sigma^2_w$ = variance among individual plants within plots

MSF = mean square of family

MSE = mean square of error

$df_F$ = degree of freedom of family

$df_e$ = degree of freedom of error

For half-sib heritability:

$$h^2_{PFM} = \frac{\sigma^2_F}{\sigma^2_{PFM}} = \frac{\sigma^2_F}{\sigma^2_F + \frac{\sigma^2_e}{r} + \frac{\sigma^2_w}{rn}}$$

$$s.e.(h^2_{PFM}) = \frac{\sqrt{\frac{2}{(rn)^2}\left(\frac{MSF^2}{df_F + 2} + \frac{MSE^2}{df_e + 2}\right)}}{\sigma^2_F + \frac{\sigma^2_e}{r} + \frac{\sigma^2_w}{rn}}$$

where $\sigma^2_A$ = additive genetic variance

$\sigma^2_{PFM}$ = phenotypic variance among half-sib family means

$\sigma^2_F$ = family variance component

$\sigma^2_e$ = error variance component or plot-to-plot environment variance

$\sigma^2_w$ = variance among individual plants within plots

r = number of replicates

n = weighted harmonic mean of number of plants in each plot

However, the assumption that phenotypic variance is constant is conservative and generally not recommended with good computing tools (Nyquist and Baker, 1991). Without assumption constraint, parametric bootstrap was used to estimate the standard error of heritability (Efron and Tibshirani, 1993). The heritabilities were estimated from observations of re-sampled families with a replacement for 1000 times. This computation is conducted in R version 3.3.2 using “sample” and “loop” functions.

Based on the heritability estimate of KL from the first estimation, in which each tray contained 17 or 18 half-sib progenies (112 progenies in total) with three replicates, the simulation by varying number of seedlings per half-sib (sample size)

and a number of replicates was conducted to determine the numbers to improve the estimation.

**Correlation of BLS between seedlings in greenhouse and mature plants in the field**

The selected CIR and ST in 2015 and 2016 were planted in a seed production nursery in Ithaca, NY (Chapter 3). The field evaluation of mature plants was conducted in August 2015 and 2016. The rating scores ranged from 0 (resistant) to 5 (susceptible) (0 = 0% BLS, 1= 1-10% BLS, 2= 11-25% BLS, 3= 26-50% BLS, 4= 51-75% BLS, 5= 76-100% BLS) (Figure 2.4). The correlations between BLS in seedlings in PLC and BLS in mature plants were computed.

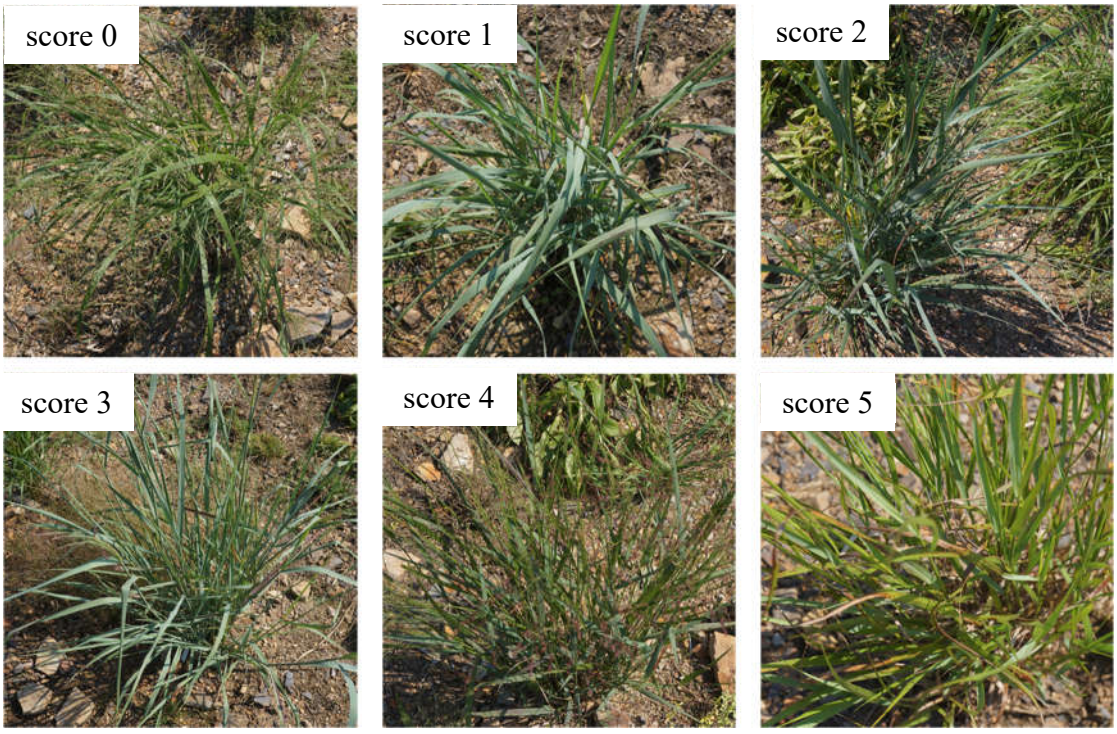



Figure 2.4. Rating of field evaluation ranges from 0 = 0% BLS, 1= 1-10% BLS, 2= 11-25% BLS, 3= 26-50% BLS, 4= 51-75% BLS 5= 76-100% BLS

**Determine non-destructive BLS evaluation in mature plant**

Since leaf detachment assay is a general term for leaf sampling via excision technique, in this research, 'detached leaf assay' is referred to trimming the leaf just on the tip and the base whereas 'leaf disk' is referred to coring the leaf into a small circle, which includes the midrib in the center. Detached leaf assay for BLS in switchgrass was explored by Fajolu (2012). This study also conducted a validation to determine the potential for three leaf detachment assay including inoculum drop, agar, and spray. The KL mature plants were the same KL plants for producing half-sib seeds. The KL seedlings were inoculated at $10^3$ or $10^4$ conidia·mL$^{-1}$ concentrations and evaluated for PLC. After transplanting to 0.3-m pots and maintaining in a greenhouse, six-month-old KL plants were sampled from their leaves. The leaves were then cut into 5-cm long pieces and surfaced sterilized by 95% ethanol for 1 minute, 10% sodium hypochlorite for 1 minute and three times of deionized water for 1 minute. Three leaves were then patted dry and placed in a petri-dish with a water-soaked filter paper. Three different inoculation techniques were applied to the leaves. First, with inoculum drop, 10 microliters of $10^5$ conidia·mL$^{-1}$ was dropped on the midrib of the each cut leaf. Second, inoculum agar was done by placing a cored PDA, which was subcultured with BO for three weeks, in the middle of each leaf (Figure 2.5a and b). Third, inoculum spray was done by spraying $10^5$ conidia·mL$^{-1}$ inoculum on each cut leaf by an airbrush at 10 psi (Figure 2.5c and d). After inoculation, all Petri dishes were sealed with paraffin and kept at 25°C under the 12-h light. The percentage of leaf

area covered with lesions was evaluated at 7 dpi, and the correlation with PLC in seedlings was computed.

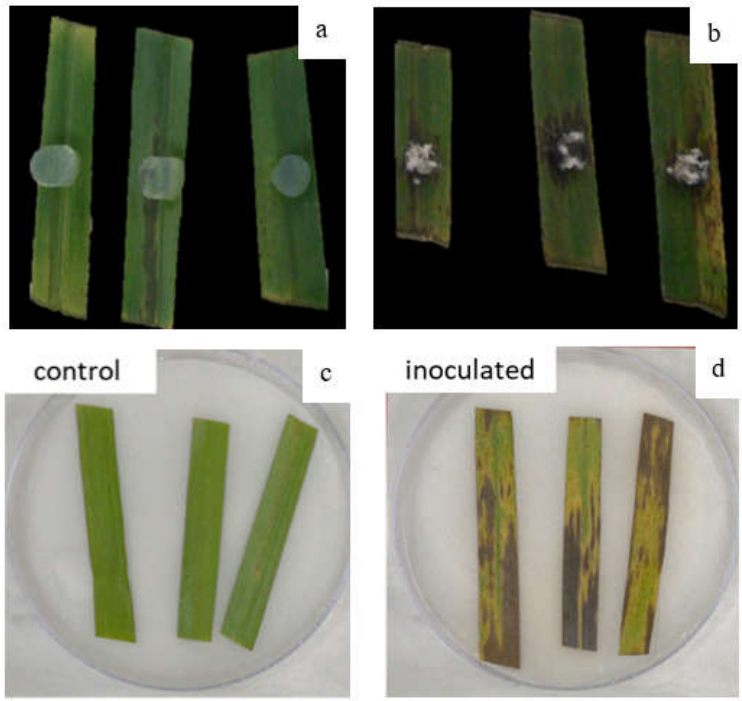



Figure 2.5. Leaf detachment assays from mature KL plants. Agar (top) and spray (bottom) assay were evaluated at 7 dpi comparing between control (a and c) and inoculated (c and d) leaves.

### Repeatability of detached leaf assay by spraying inoculation

The detached leaf assay by spraying was tested for its repeatability in mature plants from the Northern Association Panel. The repeatability was tested within and among experiment sets. Eighty-five genotypes from 479 total individuals were selected to represent all of the ecotypes. The same age or same height leaves were cut, washed with deionized water, packed in a plastic bag, put in a cooler in the field, and kept in a refrigerator overnight. Inoculum of $10^5$ conidia·$mL^{-1}$ was prepared the next day. The leaves were patted dry, inoculated, and kept in the same condition mentioned above. The repeatability was from the randomized, complete block design in three different experiment sets. Briefly, there were three leaves on each plate, and each experimental set had three replicated plates. Each replicate was placed in three different sections on the table in the laboratory. There were five control plates without inoculation in each section. The place of each plate in each section was randomized. The lesion percentages were evaluated at seven dpi both visually and by image analysis from scanning pictures from a flatbed scanner (Canon CanoScan LiDE 700F) at a resolution of 1,200 dpi by ImageJ (the code provided in the Appendix). The repeatability was tested in R by both analyses of variance via lmer package to determine if experimental set or plate had significant effects on the severity and via rptR to determine the 'repeatability' (Stoffel et al., 2017).

### Validation of leaf disk assay

In addition to leaf detachment, leaf disk assay was an alternative for non-destructive disease evaluation in mature plants. 'SW64_05', 'SW116_01', and 'SWG39_01' from the association panel were sampled and surface-sterilized as in

detached leaf assay. The leaves were cored into an 8-mm disk, patted dry and placed on wet filter paper. Each plate consisted of three leaf disks from each of three genotypes. One microliter of 0, $10^4$, 5 x $10^4$, and $10^5$ conidia·mL$^{-1}$ was dropped on the side of the mid vein in different plates. There were three replicated plates for each concentration. The plates were sealed and kept as in the leaf detachment assay. The percentage of lesions on the disk was evaluated at seven dpi.

## Results

### Determining seed inoculation technique for BSR

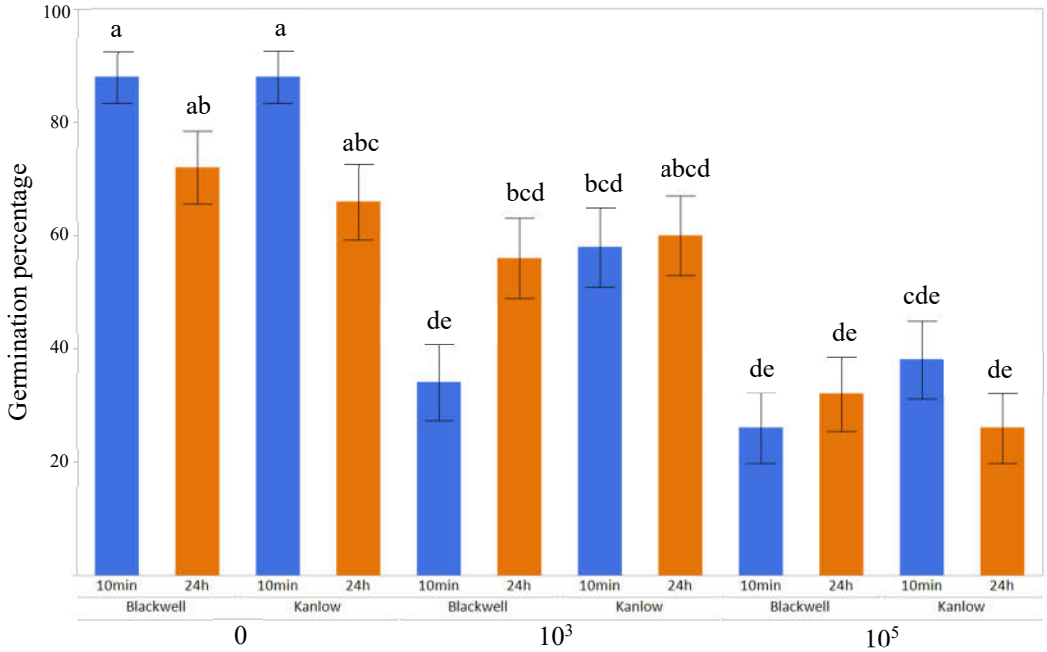
Soil inoculation showed expected numbers of germinating seeds. The control inoculum of CIR and SN has lower germinating seeds than the higher concentration of inoculum of $10^4$ conidia·mL$^{-1}$ (Table 2.1). The reduction of germination by increasing inoculum concentrations can be found in BW, but the germination remained the same in KL.

Table 2.1. Numbers of germinating seeds (out of 25 total) under different soil treatments (0, 5x$10^3$, $10^4$ conidia·mL$^{-1}$).

| **Cultivars** | **Numbers of germinating seeds (conidia·mL$^{-1}$)** | | |
|---|---|---|---|
| | **0** | **5x$10^3$** | **$10^4$** |
| CIR | 6 | 2 | 17 |
| BW | 21 | 13 | 17 |
| KL | 14 | 16 | 15 |
| SN | 2 | 2 | 7 |

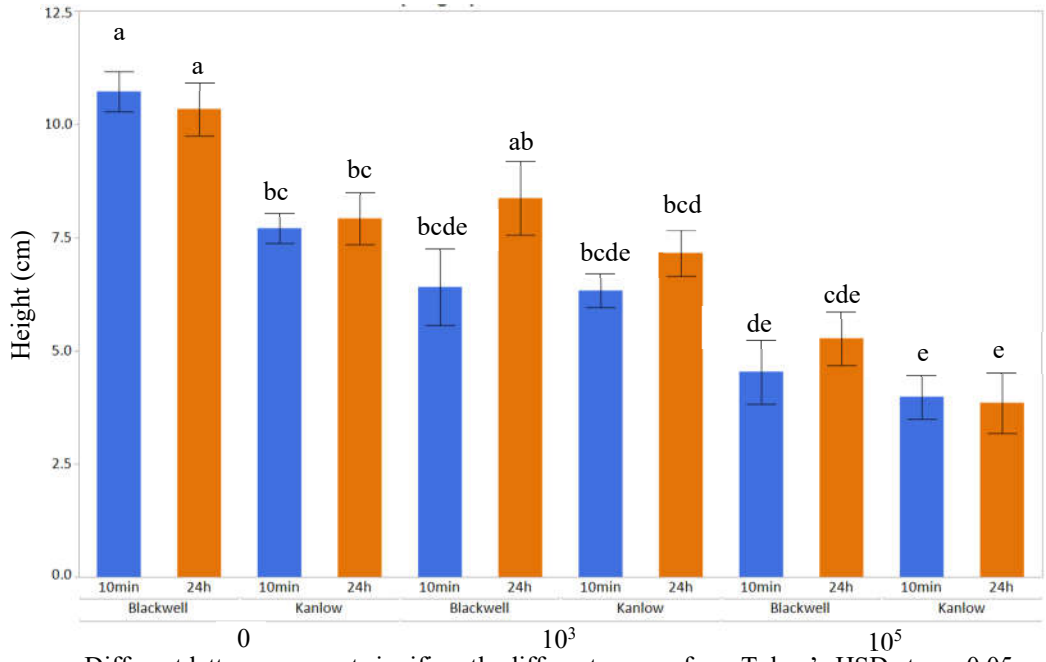
**Determining the seed inoculation condition**

In comparing the duration of seed inoculation between 10 minutes and 24 hours showed no significant different germination percentage in both BW and KL (Figure 2.6). Moreover, the higher concentration of inoculum resulted in reducing germination percentages and height in both cultivars (Figure 2.7).




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.6. Comparisons of the-fourth-week germination percentages of seed inoculation duration between 10 minutes and 24 hours shaking under 60 rpm in Blackwell and Kanlow with 0, $10^3$, $10^5$ conidia·mL$^{-1}$ inoculum concentrations.




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.7. Comparison of the-fourth-week height of seedling from seed inoculation duration between 10 minutes and 24 hours, shaking under 60 rpm, in Blackwell and Kanlow with 0, $10^3$, $10^5$ conidia·mL$^{-1}$ inoculum concentrations.

For the time series, germination percentage showed various responses to the different inoculum concentrations. The fastest germination occurred in the first week in all cultivars (Figure 2.8). In control treatment, KL showed the highest germination at 70% whereas SB showed the lowest at 4%. The effect of increasing inoculum concentration can be found in this plot also. In KL, the control provided the highest germination followed by $10^3$, $10^4$ and $10^5$ conidia·mL$^{-1}$. The germination reduction overall kept increasing until week 2. However, the germination reduction percentage started dropping at week 3 and stabilized at week four because some of the germinating seedlings died off due to the BSR.

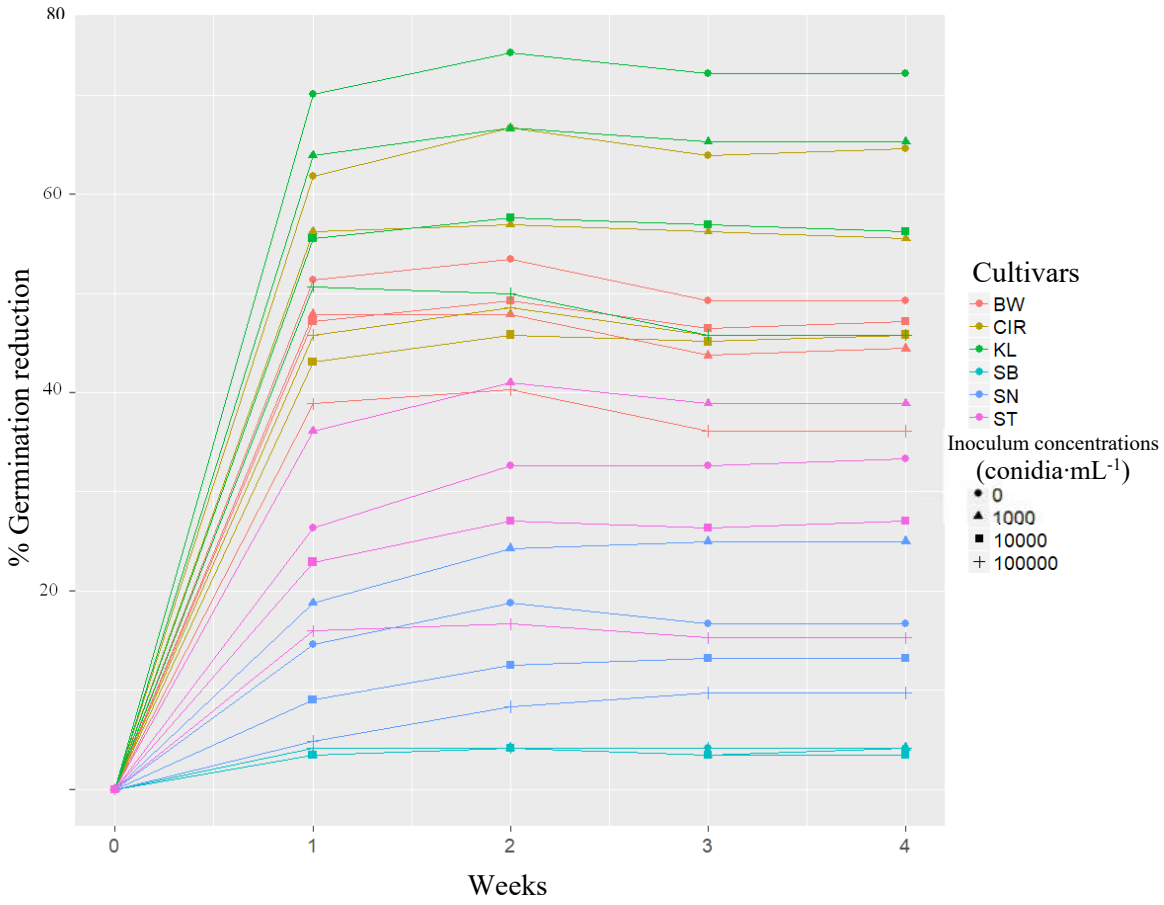
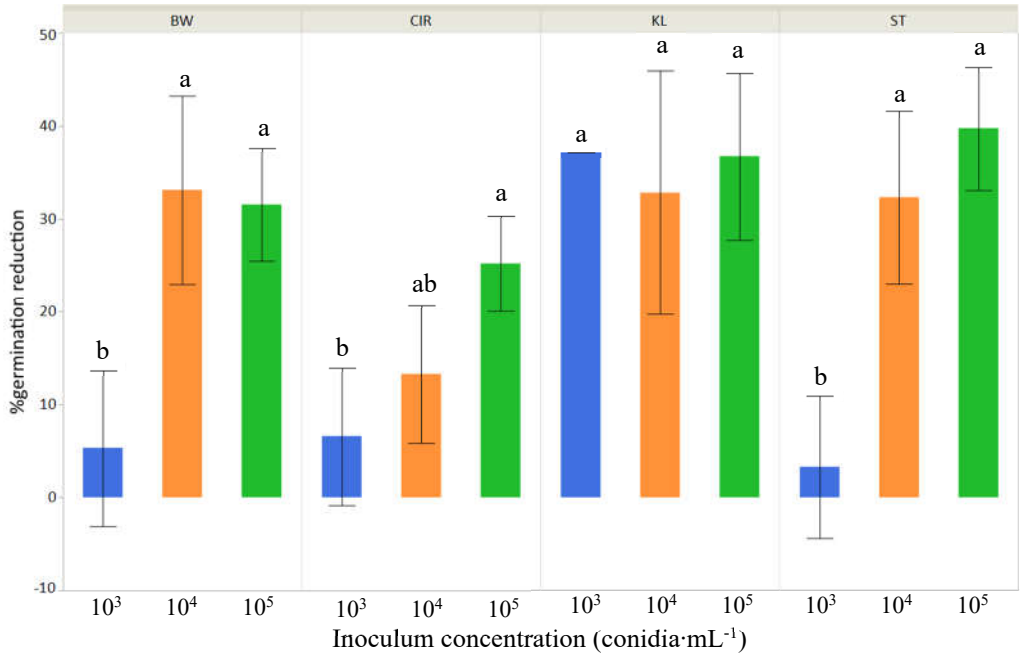



Figure 2.8. Time series of germination percentages of BW, CIR, KL, SB, SN, and ST inoculated with various concentrations of inoculum (0, $10^3$, $10^4$, and $10^5$ conidia·$mL^{-1}$).

Thirdly, the difference between germination percentage of the control treatment compared with inoculated seeds are represented as the reduction in germination percentage in Figure 2.9. In most cultivars, the concentration of $10^3$ conidia·$mL^{-1}$ showed the lowest reduction percentage although, in KL and CIR, $10^3$ conidia·$mL^{-1}$ treatment did not provide significant differences to $10^4$ conidia·$mL^{-1}$. In BW, CIR, and ST, the reduction percentages at $10^5$ conidia·$mL^{-1}$ were significantly higher than the reduction percentages at $10^3$ conidia·$mL^{-1}$.

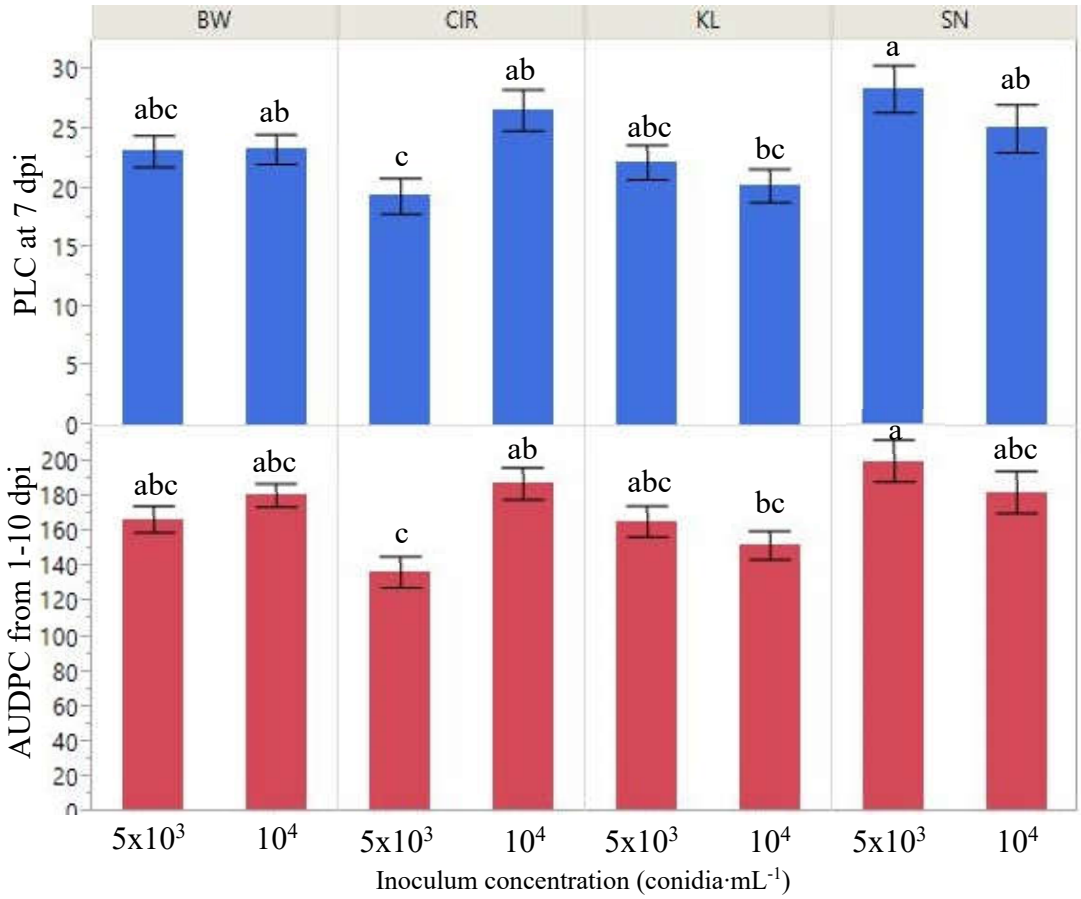



Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.9. Comparison of germination reduction percentage on week 4 by different concentrations of inoculum of $10^3$, $10^4$, $10^5$ conidia·mL$^{-1}$ in Blackwell (BW), Cave-in-Rock (CIR), Kanlow (KL) and Shelter (ST)

**Determining seedling inoculation technique for BLS**

The severity of BLS in BW, CIR, KL, and SN at both inoculation concentrations showed a similar trend regardless of whether evaluation method was PLC at seven dpi or AUDPC during 1 – 10 dpi (Figure 2.10). Such trends were confirmed by moderate to high pairwise correlations between the two approaches in all tested cultivars (Table 2.2). The PLC at seven dpi showed the highest correlations with AUDPC during 1 – 10 dpi in all tested cultivars and all concentrations.



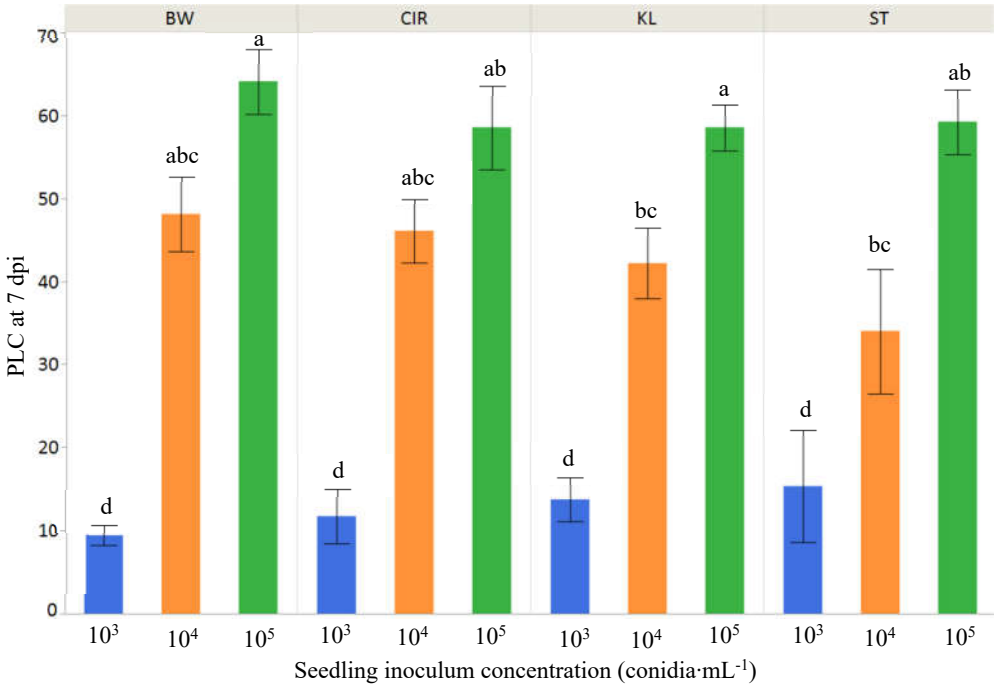

Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.10. Comparison between mean of PLC at 7 dpi and AUDPC from 1-10 dpi across cultivars (BW = 'Blackwell, CIR = 'Cave-in-Rock', KL = 'Kanlow', and SN = 'Shawnee') at 5 x $10^3$ and $10^4$ conidia·mL$^{-1}$.

Table 2.2. Pearson pairwise correlation between PLC and AUDPC at 1, 4, 7, and 10 dpi at various inoculum concentrations across various cultivars.

| Cultivars | Treatment (1000 conidia·mL$^{-1}$) | dpi | Pairwise correlation between PLC with AUDPC |
|---|---|---|---|
| BW | 5 | 1 | 0.50* |
| | | 4 | 0.89* |
| | | 7 | 0.92* |
| | | 10 | 0.85* |
| | 10 | 1 | 0.27 |
| | | 4 | 0.60* |
| | | 7 | 0.73* |
| | | 10 | 0.60* |
| CIR | 5 | 1 | 0.33 |
| | | 4 | 0.73* |
| | | 7 | 0.80* |
| | | 10 | 0.63* |
| | 10 | 1 | 0.56* |
| | | 4 | 0.59* |
| | | 7 | 0.89* |
| | | 10 | 0.74* |
| KL | 5 | 1 | 0.48* |
| | | 4 | 0.83* |
| | | 7 | 0.89* |
| | | 10 | 0.83* |
| | 10 | 1 | 0.38 |
| | | 4 | 0.67* |
| | | 7 | 0.92* |
| | | 10 | 0.84* |
| SN | 5 | 1 | 0.48 |
| | | 4 | 0.76 |
| | | 7 | 0.86* |
| | | 10 | 0.83* |
| | 10 | 1 | 0.64 |
| | | 4 | 0.64 |
| | | 7 | 0.89* |
| | | 10 | 0.75 |

The suitable concentration for screening was tested by varying inoculum concentrations. The higher concentrations showed the higher mean of PLC at seven dpi (Figure 2.11). However, there were non-significant differences between $10^4$ and $10^5$ conidia·mL$^{-1}$ in three cultivars BW, CIR, and ST. Only, KL exhibited differences significantly among concentration. Despite non-significant PLC, the most susceptible or highest score at $10^5$ conidia·mL$^{-1}$ was BW at 64%. The lowest concentration at $10^3$ conidia·mL$^{-1}$ provided a mean of PLC at 9.8% in BW, but there was no significant variability among cultivars. The non-significantly variability among cultivars was also present in $10^4$ and $10^5$ conidia·mL$^{-1}$.




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.11. Comparison of PLC in cultivars BW, CIR, KL and ST at inoculum concentrations of $10^3$, $10^4$, and $10^5$ conidia·mL$^{-1}$.

**The effects of BLS on weight and height**

The PLC at seven dpi showed a similar result to that from determining inoculum concentration in that the concentrations of $10^4$ and $10^5$ conidia·mL$^{-1}$ were not significantly different in BW and CIR but were in KL (Figure 2.12). The dry weight of above ground biomass from either $10^4$ or $10^5$ conidia·mL$^{-1}$ was not significantly different from control seedlings in all cultivars. However, the $10^4$ conidia·mL$^{-1}$ showed significantly higher dry weight at 0.12 g than the control seedlings at 0.06 g. Such an unexpected increment was not apparent for height since none of the treatments across cultivars showed a significant reduction in height except the significant reduction in KL. Overall, the height was significantly negatively correlated with PLC at $r = 0.11$ and positively correlated with weight at $r = 0.27$ (Figure 2.13). However, the correlation between PLC and weight was not significant at $r = 0.02$.

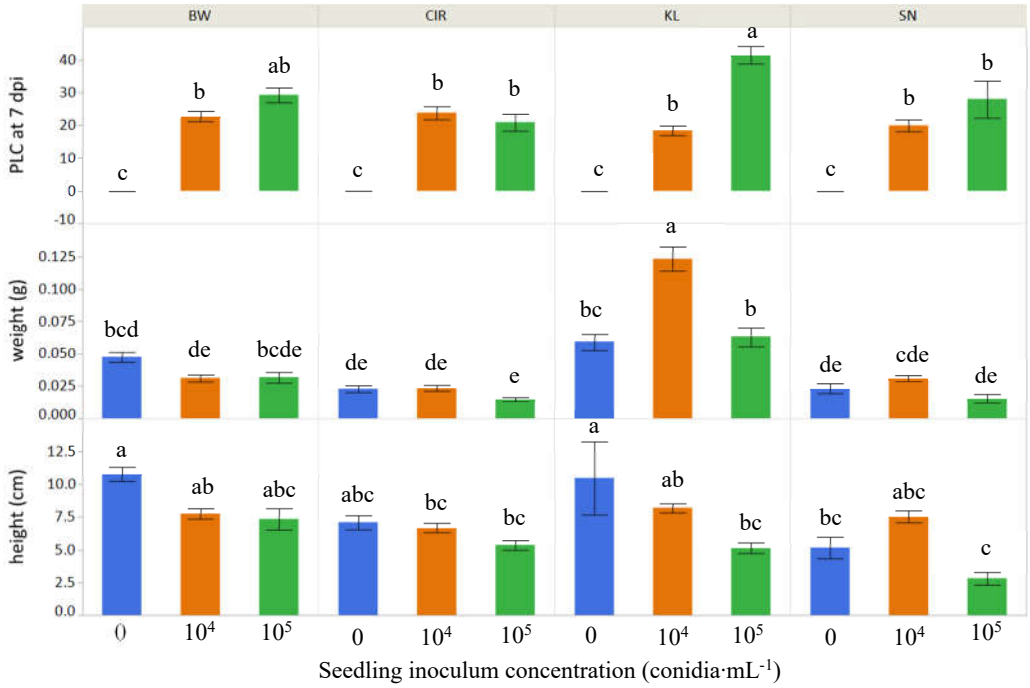
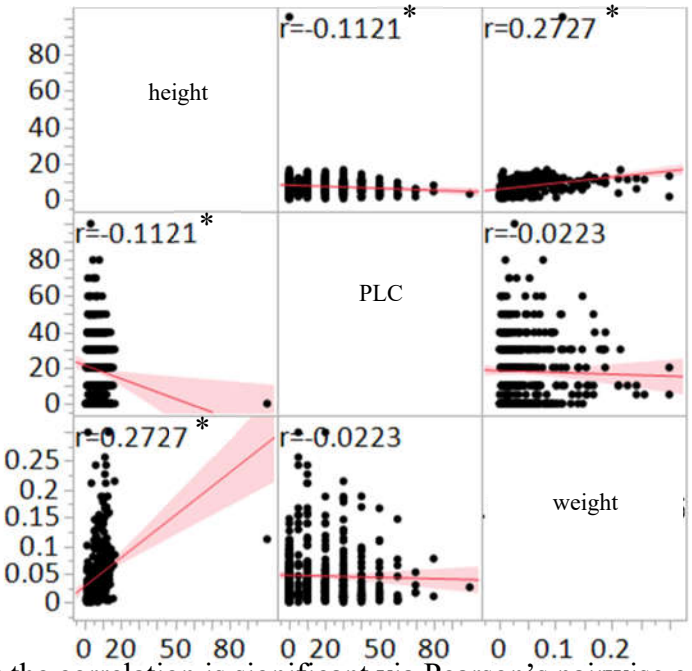



Figure 2.12. Responses of seedlings from seedling inoculations at various conidia concentrations (0, $10^4$, $10^5$ conidia·mL$^{-1}$) as PLC at 7 dpi, weight (g), and height (cm) in BW, CIR, KL and SN.

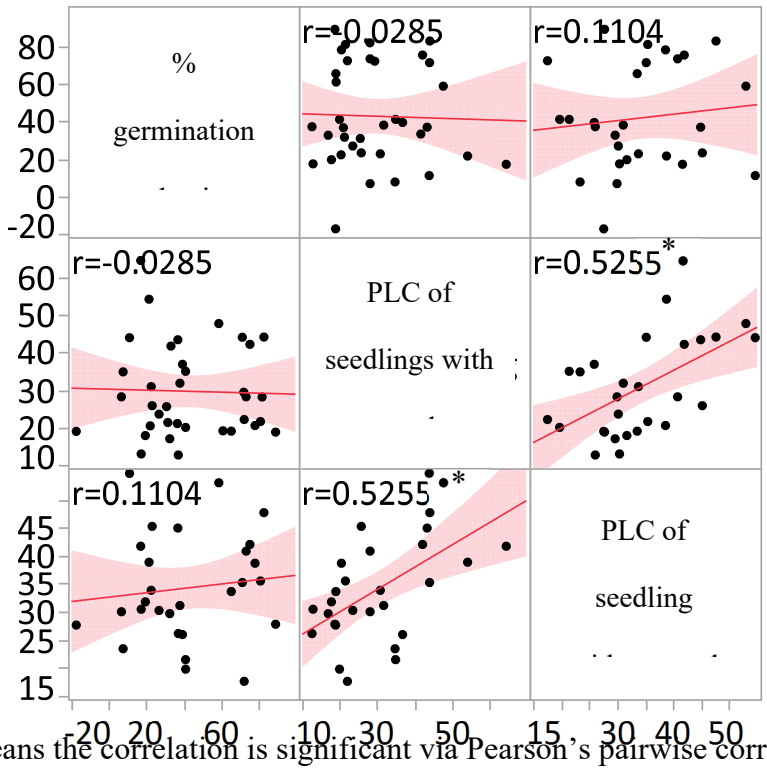



* means the correlation is significant via Pearson's pairwise correlations.

Figure 2.13. Pearson's pairwise correlations among height, weight, and PLC at 7 dpi from seedling inoculation across cultivars (BW, CIR, KL and SN) and concentrations (0, $10^4$, $10^5$ conidia·mL$^{-1}$).

**Correlation between BSR and BLS**

The correlations among 1) seed inoculation for BSR as the percentage reduction of germination, 2) seedling inoculation for BLS as PLC of seedlings that was from non-inoculated seeds, and 3) seedling inoculation for BLS as PLC of seedlings that was from inoculated seeds were from eight experiments. Across inoculum concentrations and cultivars, there were no significant correlation between percentage reduction of germination and PLC from seedlings that was from either non-inoculated or inoculated seeds at r = -0.3 and r = 0.11, respectively (Figure 2.14). Whereas, the correlation between PLC from seedlings that was from non-inoculated and inoculated seeds was significant at r = 0.53.

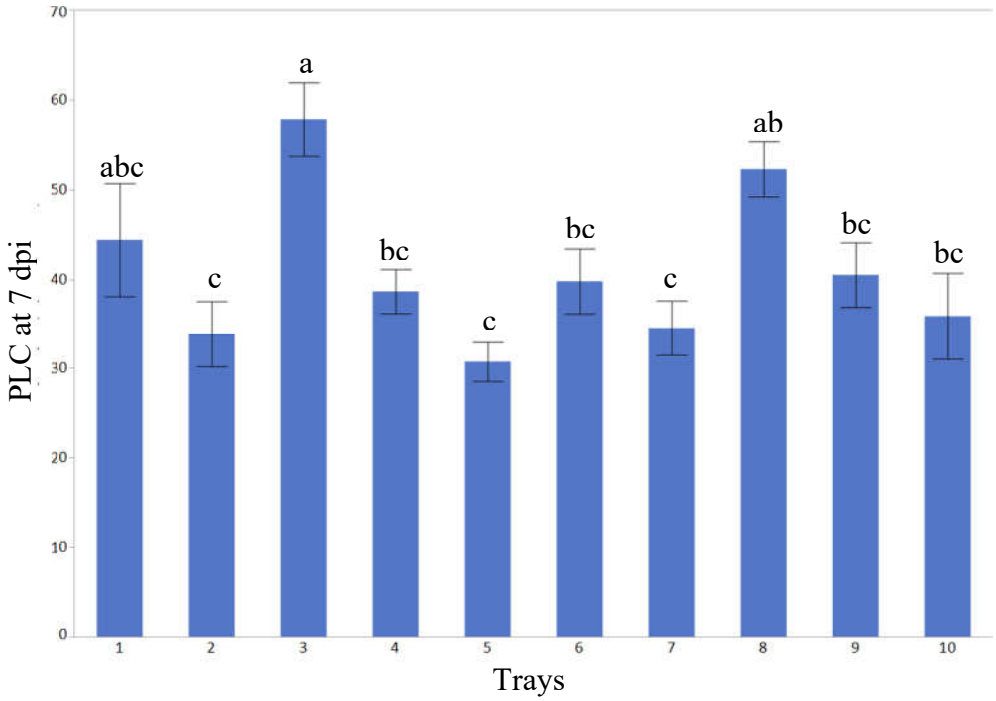



* means the correlation is significant via Pearson's pairwise correlations.

Figure 2.14. Pearson's pairwise correlations among percentage of germination reduction, PLC of seedlings with seed inoculation, and PLC of seedling without seed inoculation across cultivars (BW, CIR, KL, and SN), concentrations ($10^3$, $5x10^3$, $10^4$, $10^5$ conidia·mL$^{-1}$), and eight experiments.

**Tray-tray variation**

The severity of BLS as PLC at seven dpi of BW across ten trays confirmed that there was a tray-tray variation (Figure 2.15). Only seven out of ten trays showed non-significantly different PLC ranging from 28% to 57%.




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 2.15. Variation of PLC among trays of BW.

**The improvement of inoculation technique for heritability estimate**

In the tray arrangement of fitting half-sib progenies across multiple trays per replicate, none of the heritability estimates in either CIR or KL from inoculation by a spray bottle had a significant value from zero (Table 2.3). Based on this inoculation technique and condition, the simulation of 112 half-sib progenies of KL showed that at three replicates, the sample size of 20 seedlings would provide individual-based narrow-sense heritability of only 0.09 (Figure 2.16). In case of increasing number of replicates, 20 replicates was predicted to yield heritability of only 0.35. As such, the application method of inoculum was changed to airbrush with a control amount of inoculum and pressure. However, the airbrush with the same tray arrangement for seedlings persisted to yield non-significant heritability estimates in both CIR and KL.

The tray arrangement was changed to fit 47 half-sib progenies in one tray with five replicates. Although the heritability estimates in both CIR and KL on both half-sib- and individual-based increased in experiment 3, 4 and 5, the standard errors were higher than two times of heritability, resulting in non-significant heritabilities.

Table 2.3 Heritability estimates across multiple approaches (a spray bottle and airbrush) with various tray designs in CIR and KL.

| Cultivars | Inoculation technique | Tray | Experiment | Heritability based on | |
|---|---|---|---|---|---|
| | | | | half-sib | individual |
| CIR | spray bottle | across multiple trays per rep | 1 | 0.24 ± 0.23 | 0.12 ± 0.15 |
| | air brush | across multiple trays per rep | 2 | 0.00 ± 0.11 | 0.00 ± 0.04 |
| | air brush | rep within tray | 3 | 0.00 ± 0.07 | 0.00 ± 0.01 |
| | air brush | rep within tray | 4 | 0.32 ± 0.22 | 0.09 ± 0.08 |
| | air brush | rep within tray | 5 | 0.29 ± 0.24 | 0.11 ± 0.12 |
| KL | spray bottle | across multiple trays per rep | 1 | 0.00 ± 0.01 | 0.00 ± 0.00 |
| | air brush | across multiple trays per rep | 2 | 0.00 ± 0.12 | 0.00 ± 0.02 |
| | air brush | rep within tray | 3 | 0.27 ± 0.21 | 0.03 ± 0.03 |
| | air brush | rep within tray | 4 | 0.17 ± 0.19 | 0.06 ± 0.08 |

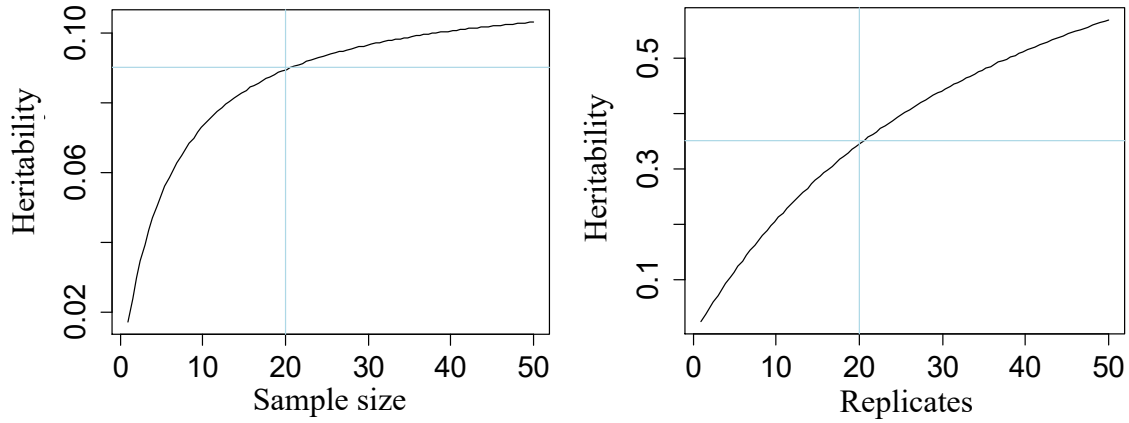



Figure 2.16. Simulation of individual-based heritability by varying sample sizes and replicate numbers of 112 half-sib progenies of KL.

**Correlation of BLS between seedlings in greenhouse and mature plants in the field**

In each year of 2015 and 2016, neither CIR nor ST showed significant correlations of resistance to BLS between seedlings and mature plants (Table 2.4). However, across both years, the correlation of both CIR and ST showed significantly low correlation at r = 0.16 and 0.23, respectively.

Table 2.4. Correlation coefficients between greenhouse and field evaluations in CIR and ST for two years.

| Cultivars | Years | Pairwise correlations | p-values | N |
|---|---|---|---|---|
| CIR | 2015 | 0.0728 | 0.2535 | 248 |
| | 2016 | 0.0961 | 0.2032 | 177 |
| | All | 0.1587 | 0.0016* | 393 |
| ST | 2015 | -0.0334 | 0.6901 | 145 |
| | 2016 | -0.0609 | 0.4640 | 147 |
| | All | 0.2296 | 0.0001* | 324 |

* means the correlation is significant via Pearson's pairwise correlations.

**Determine leaf detachment assay approach**

The KL that was inoculated in a seedling stage were used not only to produce half-sib seeds for heritability estimates but also to compare the leaf detachment

approaches among inoculum drop, agar, and spray. At seven dpi, the mean PLC from the various inoculation approaches were not significantly different, except PLC from spraying inoculation was significantly correlated to PLC in seedlings at r = 0.24 (Table 2.5).

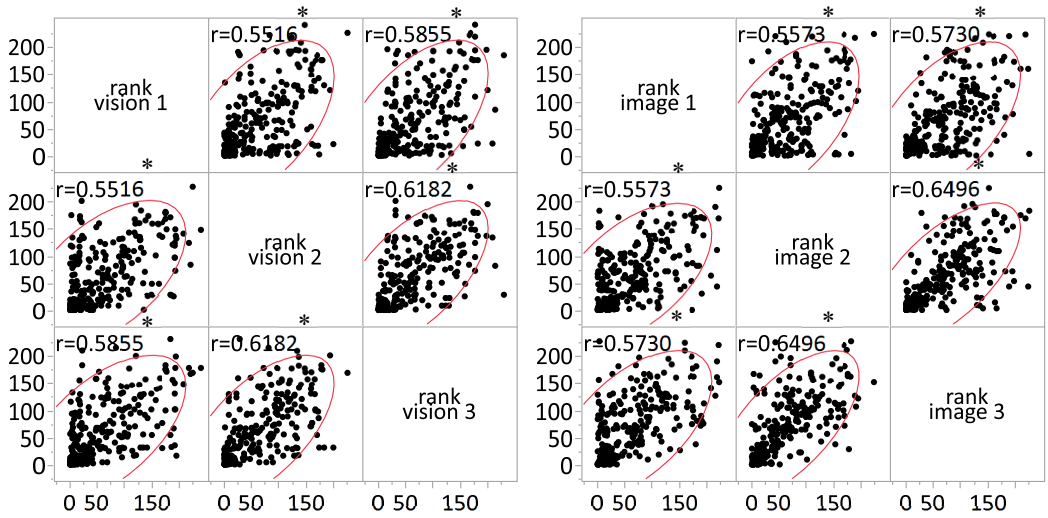
Table 2.5. Mean PLC and correlation between greenhouse evaluation of KL seedlings and leaf detachment assays (inoculum drop, agar, and spray) from the mature KL.

| Treatments | PLC at 7 dpi | Correlation | p-value | N |
|---|---|---|---|---|
| drop | 35.5 | 0.01 | 0.96 | 22 |
| agar | 26.3 | 0.18 | 0.13 | 73 |
| spray | 36.8 | 0.24 | 0.026* | 84 |

* means the correlation is significant via Pearson's pairwise correlations.

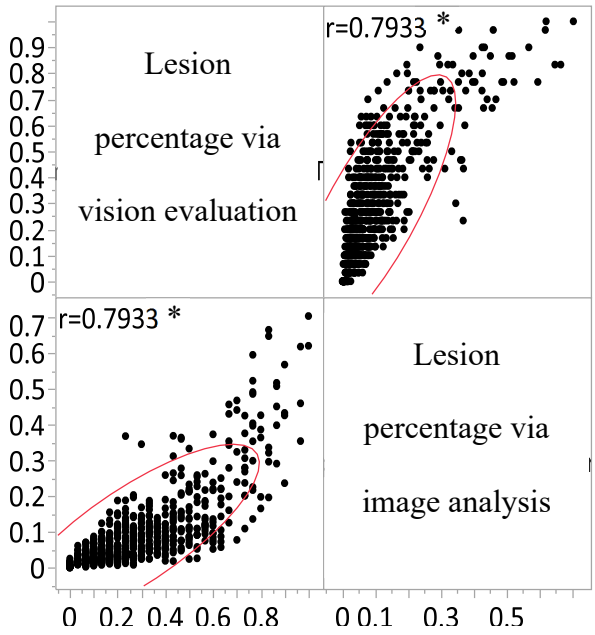
### Validation of detached leaf assay via spraying inoculation

Analysis of variance showed that the variation within the experiment (plate) was not significant, but the variation among experiments was significant. This was also confirmed via the repeatability of the experimental set being 0.03 (s.e. = 0.03). However, the repeatability of the plate was 0.00 (s.e. = 0.001). The variability among experimental sets was also confirmed by the inconsistency of the rank of severity among experiments (Appendix). The rank of genotypes among three experiments correlated only at about 50% (Figure 2.17). Despite the high variation among experiments, the evaluation via vision and image analysis had a high correlation at r = 0.79 (Figure 2.18).




* means the correlation is significant via Pearson's pairwise correlations.

Figure 2.17. Correlation of lesion percentage from leaf detachment assay by spraying among three experiment sets via vision evaluation (left) and image analysis (right).




* means the correlation is significant via Pearson's pairwise correlations.

Figure 2.18. Correlation between lesion percentage from vision evaluation and image analysis from leaf detachment assay by spraying $10^5$ conidia·mL$^{-1}$-inoculum across 85 genotypes, 3 replicates (plates) and 3 experimental sets.

**Validation of leaf disk assay**

At seven dpi, the leaf disks showed necrotic lesions and senescence (Figure 2.19). Analysis of variance showed no significant variations among replicated plates or within replicates in the plate. This was also confirmed via repeatability value of the plate at 0.35 (s.e. = 0.12). The replicated leaf disks showed high variation potentially due to an unexpected phenotype of the response to BLS. The leaf disk assay was expected to have a variation of the lesion size expanding from the inoculation site. However, the result of this experiment was total disk necrosis. This necrosis was confirmed that it was from BO by comparing with a control plate (Figure 2.19) and the validity of inoculum on PDA (Figure 2.20).

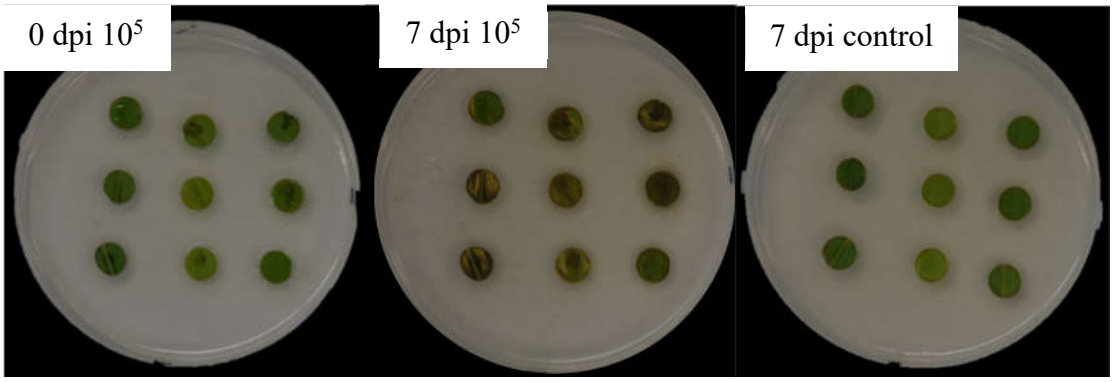



Figure 2.19. Disease severity at 0 dpi $10^5$ conidia·mL$^{-1}$ inoculum (left), 7dpi $10^5$ conidia·mL$^{-1}$ inoculum (middle) and 7 dpi uninoculated control from leaf disk assay of 'SW64 05', 'SW116 01', and 'SWG39 01' (each column).

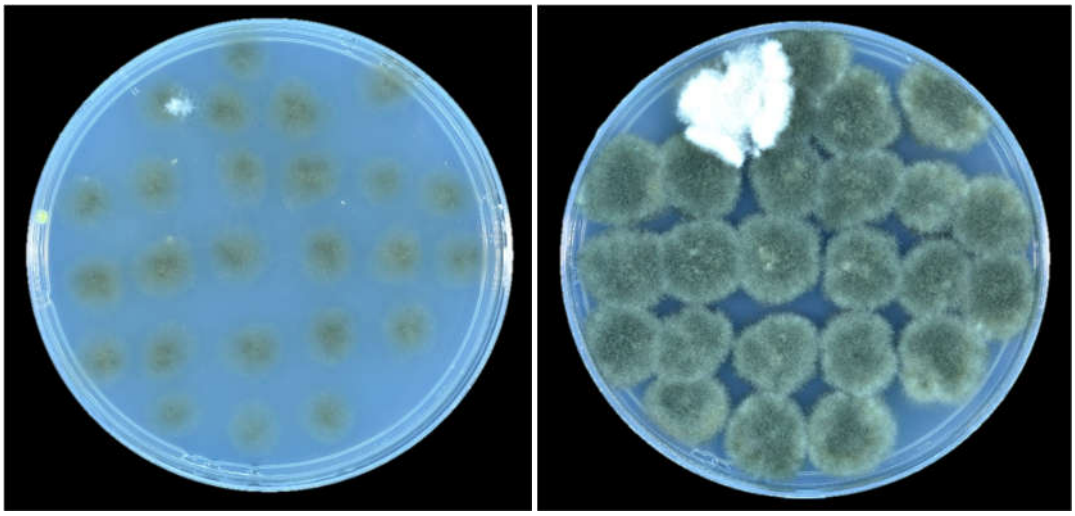


Figure 2.20. Validation of the presence of *Bipolaris oryzae* inoculum on PDA at 3 dpi (left) and 7 dpi (right) for the leaf disk assay.

**Discussion**

The screening technique for resistance to BSR was more reliable when using seed inoculation because the soil inoculation with conidia suspension did not provide a consistent result in seed germination. The inconsistency of the soil inoculation potentially came from the short incubation time when the inoculum was first inoculated to the soil. The short-incubated inoculum can also be rinsed out by the water in the next day of daily watering. This soil inoculation was also different from another BO study in switchgrass where they used dried BO colonized barley grains as inoculum to the soil (Fajolu, 2012). Therefore, in my research seed inoculation was selected for the screening method for BSR. Since there was no difference between 10 minutes and 24 hours in inoculation, which was the modification from Fajolu (2012), the standard inoculation time was determined at 10 minutes, followed by allowing the inoculum to dry on the surface for overnight to firmly attach to the seeds. The time to collect the seed germination data was determined at week 4. Although week 3

provided the highest germination, some of the germinating seedlings died due to subsequent BSR within a week after week 4, which was similar to Fajolu (2012). Lastly, the concentration of the inoculum was determined at $10^5$ conidia·mL$^{-1}$, which affected the germination the most, to screen for the resistance to BSR. The suggestions to use the high disease pressure to progress the selection were common across crops (Russell, 2013; Van Der Merwe et al., 2013).

The screening for BLS was challenging due to the variation among the inoculation and evaluation techniques. First, the score for evaluating BLS was determined as PLC at seven dpi due to high correlation with AUDPC during 1 – 10 dpi. The scoring by PLC instead of AUDPC was beneficial to the screening to be able to handle a greater population size with limited labor. The PLC on a specific day post inoculation was also used in other studies (Torres and Teng, 1993; Fajolu, 2012). The concentration to screen for BLS was selected at $10^5$ conidia·mL$^{-1}$. Although $10^5$ conidia·mL$^{-1}$ did not provide significantly higher PLC than $10^4$ conidia·mL$^{-1}$, it showed the higher mean of PLC. The effects of BLS on seedling height and weight in this experiment were not significantly different between control and the various concentrations. These results were not consistent with Fajolu (2012) because of the different inoculation technique, stage of sampling plants, cultivars, and sample sizes. More replicates with bigger sample size at an older stage of plants were required to determine the effects of BLS.

The correlation between resistances to BSR and BLS was not significant. The mechanism of infection in seed for BSR and seedling of BLS was potentially different for disease development. This lack of correlation provided the basic knowledge to

design the breeding program by making selection separately with an expectation that either type of resistance will not have a negative genetic drag on the other.

Since the selection for BLS was conducted from thousands of seedlings, the screening needed to be done in multiple trays. However, due to tray-tray variation, I recommend considering tray effects in an index selection, using best linear unbiased prediction (BLUPs), or selecting the best plants from each tray like in a grid selection system. The tray design and seedling arrangement in each tray also had effects on heritability estimates. Although the improvement in tray design and inoculation technique did not provide significant heritability estimates in KL and CIR half-sib progenies, heritability was improved by reducing variations from trays and inoculation techniques. The improvement of inoculation techniques was applied to selection for BLS. In the first year of selection, the inoculation was conducted via a spray bottle with high variability. With an airbrush, the inoculation in the second year provided more consistency of the amount and the finer droplets on leaves.

It was important to notice that the resistance to BLS in the greenhouse was not transferable to the resistance in the field based on the selected population of CIR and ST. The inconsistency across greenhouse and field is common across crops and their diseases (Foolad et al., 2000; Twizeyimana et al., 2007). However, the non-destructive disease evaluation from mature plants was still crucial. In the leaf detachment assay, spraying inoculation was the only inoculation technique significantly correlated to severity of BLS in the seedlings. In this experiment, the inoculum drop was not reliable due to the uncontrollable dispersal of inoculum when dropped. This could be improved in the future by drying the leaf surface thoroughly, adding more surfactant,

and drying the inoculum on the surface before sealing the plates. The BO agar inoculation was also not reliable due to the inconsistency of the number of hyphae and conidia in the same agar size. Despite high correlation with resistance to BLS in KL seedlings, the leaf detachment by spraying inoculation showed inconsistency among experimental sets. The high variation could have been from different leaf stages even though the same position of the leaves was sampled from the same plant. The consideration of experimental effect as a fixed effect can help lessen the problem via BLUPs. In addition to consistency of detached leaf, the evaluation could be done via image analysis due to high correlation with the evaluation of vision and the ability to trace back and reanalyze the pictures.

Another non-destructive assay was leaf disk assay. The repeatability among plates was acceptable, but I suggest including the checks in every plate in future experiments. The whole disk necrosis instead of the dot expansion could be explained via one of the mechanisms of BO infection in that the BO controls the ethylene production in the host, resulting in senescence and a benefit to the fungus (Van Bockhaven et al., 2015).

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# CHAPTER 3

## RESISTANCE TO BIPOLARIS SEED ROT AND LEAF SPOT


### Abstract

Switchgrass (*Panicum virgatum* L.) is a biomass candidate crop which has been improved for yield, but disease resistance development has largely been ignored. *Bipolaris oryzae* (Breda de Haan) Shoemaker can cause Bipolaris seed rot, reducing seedling establishment, and Bipolaris leaf spot, reducing biomass yield. Before initiating a breeding program, heritability estimates were conducted in half-sib progenies of lowland 'Kanlow' and upland 'Cave-in-Rock'. Test crosses in upland 'Shelter' and Cave-in-Rock were done to determine genetic control of resistance to Bipolaris leaf spot. Recurrent phenotypic selection for resistance to two diseases was done separately in Shelter and Cave-in-Rock. As a result, resistance to Bipolaris seed rot showed moderate to high heritability in both individual- and half-sib-based computations, reflecting actual genetic gain from selection. However, resistance to Bipolaris leaf spot showed non-significant heritability and non-significant progress of selection for the trait. Results from test crosses suggested that the resistance was not controlled by R genes but possibly was quantitatively inherited by many alleles with small effects. Such a difference in the gains resulted in non-significant correlation between two resistances. Due to high progress, the selection method for resistance to Bipolaris seed rot can be integrated to existing breeding programs.

## Introduction

Switchgrass (*Panicum virgatum* L.) has been selected as one of the potential bioenergy crops by the U.S. Department of Energy (DOE) (McLaughlin and Kszos, 2005b). Yield improvement and agronomic traits were the main target for breeding (McLaughlin and Kszos, 2005b). Even with such improvement, the highest yield of switchgrass is 11.1 ton/ha/year, which cannot meet the ideal yield requirement to serve a current demand at 50 ton/ha/year (Henry, 2010). To maintain the capacity to feed sufficient biomass, alleviating yield loss is a supplement to improving yield. One of the major yield losses in switchgrass is from diseases. Bipolaris seed rot (BSR) and Bipolaris leaf spot (BLS) are diseases caused by *Bipolaris oryzae* that can reduce biomass yield up to 50% and germination up to 74% (Fajolu et al., 2012).

Switchgrass is an open pollinated crop with high self-incompatibility. Based on such a population structure, recurrent selection is commonly used to improve switchgrass by exploiting additive genetic variance (Bouton, 2007). Another challenge in switchgrass breeding is the diversity of ploidy level ranging from tetraploid (4X) to dodecaploid (12X) (Lu, et al., 1998). In general, lowland switchgrasses are tetraploid and upland switchgrasses are octaploid. Despite complex ploidy level, disomic segregation suggests diploid-like inheritance (Okada et al., 2010). Moreover, switchgrass is diverse and wildly adapted across North America. The target environment for breeding is the Northeast USA because high humidity and high temperature during summer is favorable to the diseases (Zeiders, 1984).

*Bipolaris oryzae* (Teleomorph: *Cochliobolus miyabeanus*) is a necrotrophic Ascomycetes fungus. The fungus can affect switchgrass production from

establishment to annual yield. The primary inoculum is from infected seeds where the fungus can suppress seed germination and rot the shoot and root at the beginning of germination (Ou, 1985). In this research, such symptoms are collectively referred as BSR. Later in biomass production, the fungus residing in soil or crop residue can sporulate its conidia on leaves and cause a dark brown oval-shaped lesion called BLS (Fajolu et al., 2012). In rice, this disease was called as a poor-man disease due to high BLS disease pandemic in a low nutrient field. Such a low-input marginal land, which is a target area for growing switchgrass to avoid food-fuel competition, can intensify the BLS in switchgrass (Padmanabhan, 1973). To keep switchgrass price-competitive, breeding to improve resistance is more suitable than applying fungicides yearly such as Bavistin, Hinosan, Tilt 250 EC (Propiconazole), and Dithane M-45 (Ahmed et al., 2002). Resistant cultivars and QTLs of the resistance have been identified in rice (Sato et al., 2008, 2015). However, BLS resistance in switchgrass has not been studied besides pathogenicity of *Bipolaris* spp. in switchgrass (Krupinsky et al., 2004; Fajolu et al., 2012)

The objectives of this research are 1) to estimate the heritability of the resistances to BSR and BLS prior to the selection from half-sib progenies of lowland and upland switchgrass, 2) to verify the genetic control of resistance to BLS in upland switchgrass, and 3) to determine the genetic gain and correlation from selections of resistances to both diseases in upland switchgrass.

## Materials and Methods

### Bipolaris isolation and inoculation preparation

Bipolaris conidia was isolated from a 'Carthage' switchgrass leaf in the warm season biofuels field experiment in Ithaca, NY by Katie Waxman (Waxman and Bergstrom, 2011). *Bipolaris oryzae* was subcultured on potato dextrose agar. The inoculum was prepared from three-week-old plates, filtered via gauze, and adjusted to a concentration of $10^5$ conidia/ml by a hemocytometer with 2 drops of Tween-20 per 100 ml. Control solution was sterilized water and Tween-20.

### Plant material

To estimate heritabilities, half-sib seeds of Cave-in-Rock (CIR) and Kanlow (KL) were produced. To produce these seeds, 143 Cave-in-Rock plants were planted in a 0.9-meter spaced plot and allowed to be wind cross pollinated in a field nursery in Ithaca, NY, in 2014. One hundred sixteen Kanlow seeds were planted in Cornell Peat-lite media in 30-centimeter pots in a greenhouse under 12-h photoperiod of supplemental light by high-pressure sodium lamps at 40 klm and ambient temperatures of 18 to 29°C. During anthesis of Kanlow in the greenhouse (end of October to November, 2014), the potted plants were randomly arranged every day to allow random pollination within the population. All the seeds were hand-harvested between August and September for CIR and December and January for KL, dried at 55ºC, and kept in a separate bag for each half-sib seeds.

### Heritability estimates

Heritabilities of resistance to both BSR and BLS were estimated from half-sib progenies. Two switchgrass cultivars CIR and KL were used to estimate heritability as

representatives of upland and lowland switchgrass, respectively. Each tray consisted of 'Blackwell' (BW) as a susceptible check cultivar. For BSR, switchgrass seeds were surface-sterilized and dried overnight. In each half-sib progeny, seeds were separated into two groups of control and inoculated conditions. The inoculated group was soaked in inoculum of $10^5$ conidia/ml at 0.2 g of seeds per 25 ml of inoculum, shaken at 60 rpm for 10 minutes, drained the inoculum out, and dried overnight. The control group was inoculated the same way but using water with Tween. Six seeds of each half-sib were planted 1-cm depth in six cells of 24 by 12 cell trays filled with Cornell Peat-lite media. The experiment was done in three replications. The number of healthy seedlings was collected on day 28 after planting and compared with the control half-sib seedlings.

For BLS, the half-sib seeds were planted in the same type of trays without seed inoculation. Four-week-old seedlings were inoculated with 20 ml of inoculum per tray by an airbrush. Each tray was sprayed evenly under a clear plastic bag which was then covered for 24 hours in a greenhouse to increase humidity resembling natural inoculation. The trays of seedlings were kept in the greenhouse for one week with watering twice daily. The experiment was done in six replicates. Each seedling was evaluated for percentage of leaf covered with lesions (PLC) at 7 days post inoculation (dpi).

Heritability estimates were calculated on both individual and half-sib basis adjusted from Gianola (1982) and Nguyen and Sleper (1983). The standard error of heritability was estimated from bootstrap method for 1000 times (Efron and Tibshirani, 1993).

Since BSR was collected as a binary trait of germinated seeds (1) and non-germinated seeds (0) having the probability $\Pr(Y = [0/1]X)$, the trait was assumed to be controlled by normally distributed multiple alleles called latent variability l ("liability") (Gianola, 1982). The germinating seeds showing the resistance to BSR, therefore, have cumulative alleles higher than the threshold (t). That proportion expressing resistance to BSR above threshold is $\pi$ and the proportion expressing susceptible to BSR below the threshold is $1 - \pi$ (Figure 3.1). Thus, the linear mixed model for resistance to BSR is

$$\log\left(\frac{\pi_i}{1-\pi_i}\right) = \beta_0 + r_j + f_i + r_j \times f_i + g_i + \epsilon_{ij}$$

where $\beta_0$ is the intercept, $r_i$ and $f_j$, are the random effects of replicate j and half-sib family i, respectively; $r_j \times f_i$ indicates the interaction of the random effect of half-sib family and replicate; $g_j$ is the fixed effect of germination in half-sib from family i without inoculation; $\epsilon_{ij}$ are residuals.

Individual-based narrow-sense heritability is

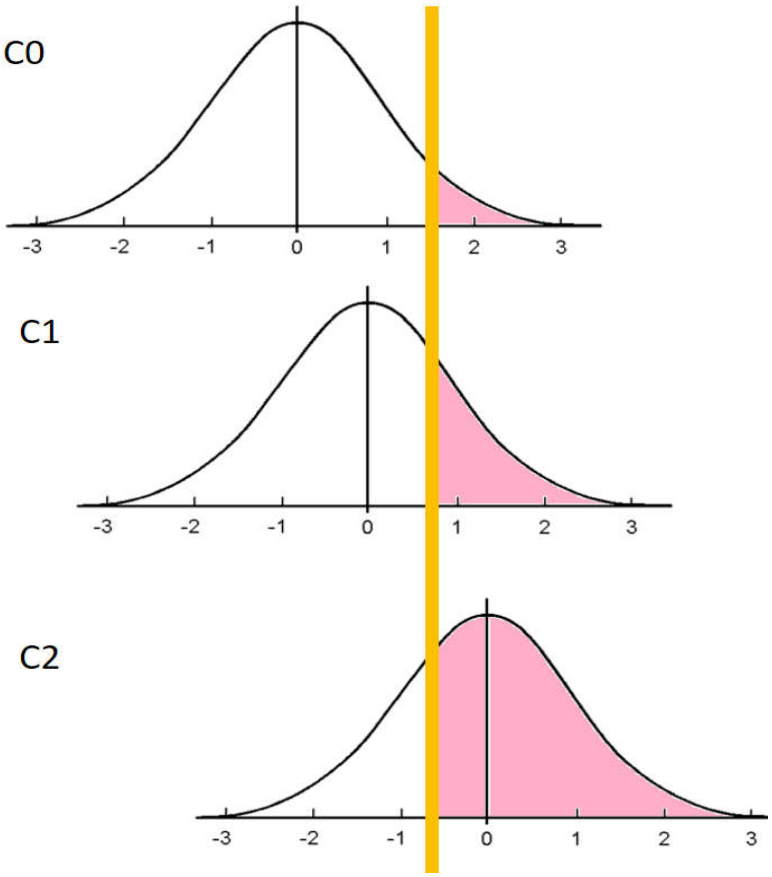
$$h^2_{individual} = \frac{4\sigma_F^2}{\sigma_F^2 + \sigma_e^2 + \frac{\pi^2}{3}}$$

Half-sib-based narrow-sense heritability is

$$h^2_{half-sib} = \frac{\sigma_F^2}{\sigma_F^2 + \frac{\sigma_e^2}{r} + \frac{\pi^2}{3rn}}$$

where $\sigma_F^2$ is variance due to half sib family; $\sigma_e^2$ is variance due to error; r is number of replicates; n is number of seeds in each half sib-replicate combination; since the resistance to BSR was measured in a binary manner of germinated or not, the

variance within half-sibs of such a logistic distribution equals to $\pi^2/3$ (Merlo et al., 2006).




Scale of liability (standard deviation from

Figure 3.1. The different expected distribution of alleles of threshold character (yellow line) from each cycle of selection; the above threshold proportion expressing resistance is π (pink area).

The following linear mixed model was fitted for resistance to BLS:

$$y_{ij} = \mu + r_j + f_i + r_j \times f_i + \epsilon_{ijkl}$$

where y is the percentage of leaf area covered by lesions, $\mu$ is the grand mean; $r_j$ and $f_i$ are the random effects of replicate j and half-sib family i, respectively; $r_j \times f_i$ indicates half-sib family effect i and replicate j; $\epsilon_{ij}$ are residuals.

Equation for individual

$$h^2_{individual} = \frac{4\sigma^2_F}{\sigma^2_F + \sigma^2_e + \sigma^2_{within}}$$

With line germination as fixed effect

Equation for half-sib

$$h^2_{half-sib} = \frac{\sigma^2_F}{\sigma^2_F + \frac{\sigma^2_e}{r} + \frac{\sigma^2_{within}}{rn}}$$

where $\sigma^2_F$ is variance due to half sib family; $\sigma^2_e$ is variance due to error; r is number of replicates; n is a harmonic mean of number of seedling in each replicate; $\sigma^2_{within}$ is variance due to within half-sib effect.

**Test crosses**

To investigate further into the genetic control of resistance to BLS, test crosses between resistant and susceptible individuals were conducted in ST and CIR. After evaluating PLC by lesions at 7 dpi, 11 and 9 most resistant and 8 and 9 most susceptible and surviving seedlings of ST and CIR, respectively, were selected and transplanted to 30-centimeter diameter pots. These plants were kept in the greenhouse and arranged into groups. Each group contained two resistant and two susceptible plants. Due to artificial photoperiod of 12-hour light, ST and CIR started to produce

panicles during late July to September, 2016. Non-anthesis mature panicles between plants in each group were bagged together in a maize pollination bag every week to produce as many resistant-resistant (R-R), resistant-susceptible (R-S), and susceptible-susceptible (S-S) crosses as possible. The bagged panicles were released approximately 4 weeks after bagging or until both panicles were pollinated. The specific crossed seeds from each panicle were then harvested 4 weeks after releasing the bags. After drying and surface sterilization, the seeds were planted in trays and tested for resistance to BLS as in heritability estimates.

**Selection**

The recurrent phenotypic selection for both resistances were done in 'Cave-in-Rock' and 'Shelter' between 2014 and 2016 in the greenhouse, and seeds of the selected plants were produced in 0.9-meter-spaced plot in a field nursery in Ithaca, NY. In each cycle of selection, an equal amount of each half-sib seeds was bulked and surface-sterilized. Selection for resistance to BSR was done for two cycles at approximately 10% selection intensity. The germinating seedlings with no signs of symptoms were selected from 1000 to 2000 inoculated seeds, depending on germination percentages. The seed inoculation was done the same way as with the heritability experiments. The genetic gain from selection(R) and liability heritability ($h_l^2$) were calculated as

$$\mathrm{R} = \mu_{Cn} - \mu_{C(n-1)}$$

$$h_l^2 = \frac{m_O - m_P}{i}$$

where $\mu_{Cn}$ is mean of germination reduction of cycle n; i is selection intensity; $m_O$ and $m_P$ is mean liability of offspring and parents, respectively (Falconer, 1966). Both 'm' are calculated from quantile of percent germination of inoculated seeds.

Selection for resistance to BLS was done for two cycles at approximately 10% selection intensity. The inoculated seedlings with the lowest PLC were selected from about 1000 seedlings. The inoculation was similar to the heritability experiments, but in the first cycle was done with a spray bottle instead of an airbrush. The genetic gain from selection and realized heritability were calculated as

$$\mathrm{R} = \mu_{Cn} - \mu_{C(n-1)}$$

$$h^2_{realized} = \frac{\mathrm{R}}{S} = \frac{\mu_{Cn} - \mu_{C(n-1)}}{\mu_{Cn\ selected} - \mu_{Cn}}$$

where $\mu_{Cn}$ is mean of PLC of cycle n; s is selection differential.

Moreover, by using the same population and making selection separately, additive genetic correlation between two resistances were computed at the end of each selection as

$$r_A^2 = \frac{CR_{BSR}}{R_{BSR}} \frac{CR_{BLS}}{R_{BLS}}$$

Where $r_A^2$ is a joint estimate of the genetic correlation; $CR_{BSR}$ is correlated response of resistance to BSR from population selected for resistance to BLS; $R_{BSR}$ is the response from selection for resistance to BSR; $CR_{BLS}$ is correlated response of resistance to BLS from population selected for resistance to BSR; $R_{BLS}$ is the response from selection for resistance to BLS;

All of the statistical analyses were conducted in R with lme4 package. The progresses of selection were tested for significant difference based on Tukey HSD at $\alpha = 0.05$.

**Results**

Heritability estimates can determine the selection approach and predict the success of selections. Since the heritabilities of both resistances were never before estimated in switchgrass, the estimates were crucial prior to the selection. For resistance to BSR, the half-sib based heritability estimates were significantly high, and the individual-based heritability estimates were also significantly moderate in both KL and CIR, respectively (Table 3.1). Despite moderate to high heritabilities of resistance to BSR in both KL and CIR, the heritability estimates of resistance to BLS were low or not significant.

Table 3.1. Individual- and half-sib-based narrow-sense heritability from half-sib seedlings and seeds of upland Cave-in-Rock and lowland Kanlow (standard error).

| | **BLS** | | **BSR** | |
|---|---|---|---|---|
| **Cultivars** | **Individual-based** | **Half-sib-based** | **Individual-based** | **Half-sib-based** |
| Cave-in-Rock | 0.07 (0.08) | 0.19 (0.19) | 0.33 (0.16) | 0.83 (0.09) |
| Kanlow | 0.02 (0.06) | 0.08 (0.15) | 0.55 (0.19) | 0.83 (0.07) |

Such a low heritability of resistance to BLS led to further study to determine the genetic control of the trait based on gene distribution from test crosses. The distribution of severity as PLC from progenies of both CIR and ST in all crosses showed almost normal distribution without multi-modal pattern, suggesting that the resistance to BLS is controlled by multiple minor-effect alleles or environmental variation (Figure 3.2 and Figure 3.3). In CIR, mean PLC from RR (31.89) was significantly lower than that from S-S, (43.01). Although there was the trend of lower PLC from SS to RS to RR, it cannot confirm the heritability of resistance to BLS in the population. Moreover, nine reciprocal crosses showed non-significant difference, suggesting no sex-linked inheritance. However, ST did not show the trend of lower PLC from SS to RS to RR (Figure 3.3). Only R-S had significantly lower mean PLC than did S-S (Table 3.2). Among nine reciprocal crosses, only R14-R4 showed the significant difference but the rest were not significantly different as in CIR.

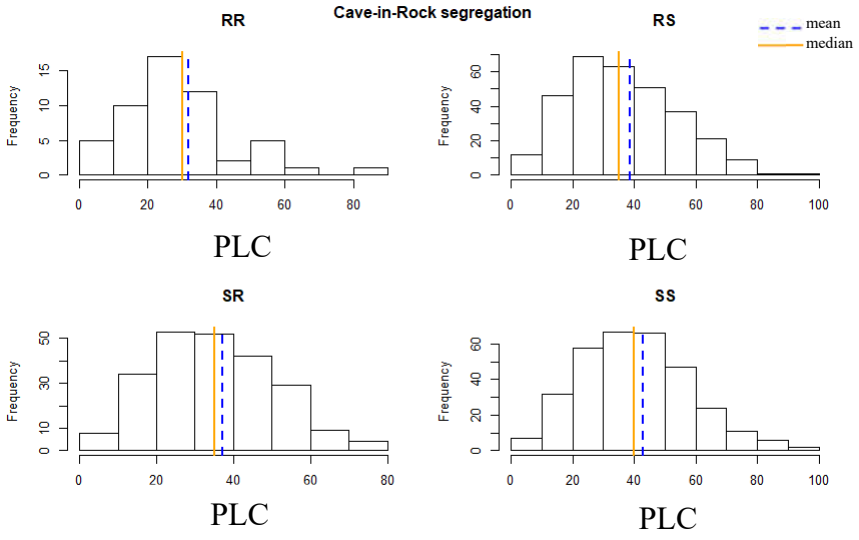



Figure 3.2. The distribution of PLC of CIR progenies from four cross types – resistance-resistance (RR), resistance-susceptible (RS), SR, and SS. Blue dashed lines represented the mean and yellow line represented the median of each cross type.

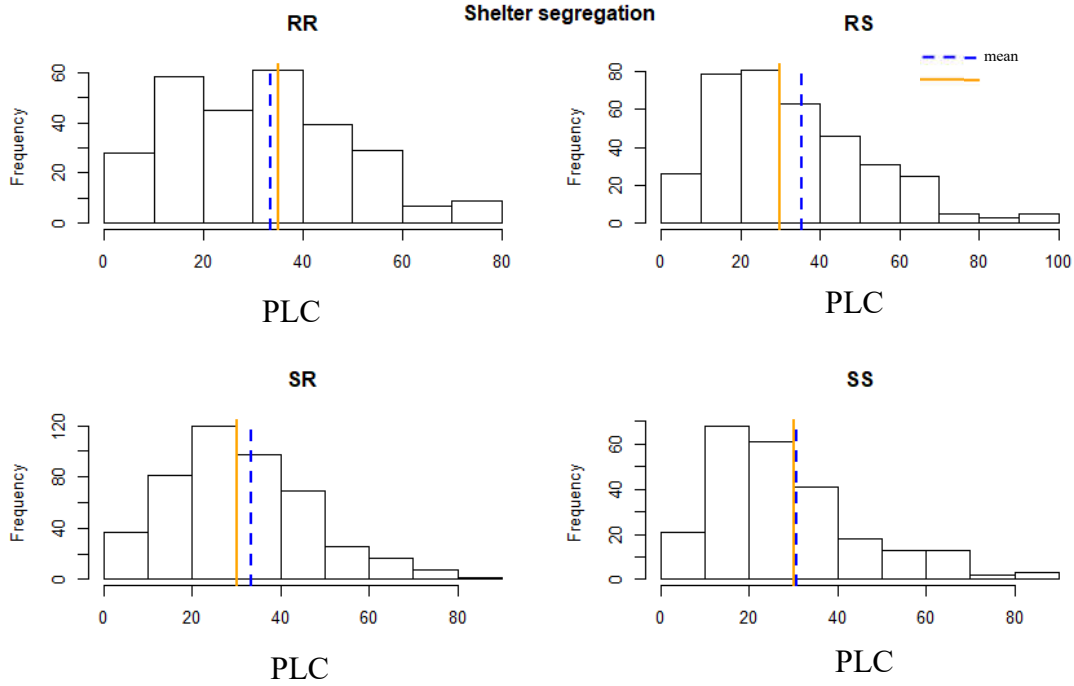



Figure 3.3. The distribution of PLC of ST progenies from four cross types – resistance-resistance (RR), resistance-susceptible (RS), SR, and SS. Blue dashed lines represented the mean and yellow line represented the median of each cross type.

Table 3.2. Mean PLC (s.e.) and median of four cross types (RR, RS, SR, SS) in CIR and ST

| | Crosses | Mean PLC (se) | Median PLC |
|---|---|---|---|
| CIR | RR | 31.89 (2.15)[a] | 30 |
| | RS | 38.52 (1.00)[ab] | 35 |
| | SR | 37.16 (1.02)[ab] | 35 |
| | SS | 43.01 (0.99)[b] | 40 |
| ST | RR | 33.64 (1.08)[ab] | 35 |
| | RS | 35.46 (1.02)[b] | 30 |
| | SR | 33.27 (0.75)[ab] | 30 |
| | SS | 30.75 (1.10)[a] | 30 |

Different superscripted letters represent significantly different group from Tukey's HSD at $\alpha = 0.05$.

Table 3.3. mean PLC (s.e.) and number of CIR progenies from specific crosses of each cross type and reciprocal crosses (shaded).

| Cross type | Female | Male | Mean PLC (s.e.) | Number of progenies |
|---|---|---|---|---|
| RR | R10 | R11 | 29.00 (4.93) | 15 |
| RR | R11 | R10 | 32.50 (7.5) | 2 |
| RR | R7 | R8 | 31.46 (3.23) | 24 |
| RR | R8 | R7 | 36.25 (3.52) | 14 |
| RS | R1 | S5 | 38.96 (2.53) | 48 |
| SR | S5 | R1 | 38.65 (2.04) | 48 |
| RS | R3 | S3 | 41.88 (2.66) | 48 |
| SR | S3 | R3 | 35.83 (2.19) | 48 |
| RS | R5 | S8 | 42.60 (2.26) | 48 |
| SR | S8 | R5 | 42.08 (2.35) | 48 |
| RS | R6 | S5 | 44.20 (2.34) | 44 |
| SR | S5 | R6 | 38.59 (2.69) | 39 |
| SS | S1 | S4 | 41.56 (2.53) | 48 |
| SS | S4 | S1 | 42.17 (3.32) | 48 |
| SS | S3 | S6 | 40.49 (2.06) | 48 |
| SS | S6 | S3 | 45.44 (2.87) | 59 |
| SS | S5 | S7 | 39.38 (2.33) | 48 |
| SS | S7 | S5 | 44.69 (2.53) | 48 |
| RS | R8 | S11 | 36.00 (3.56) | 30 |
| RS | R5 | S13 | 37.05 (2.94) | 44 |
| RS | R4 | S4 | 28.33 (1.86) | 48 |
| SS | S4 | S1 | 42.17 (3.32) | 30 |
| SR | S1 | R4 | 30.94 (1.96) | 48 |
| SS | S10 | S11 | 47.20 (2.7) | 41 |

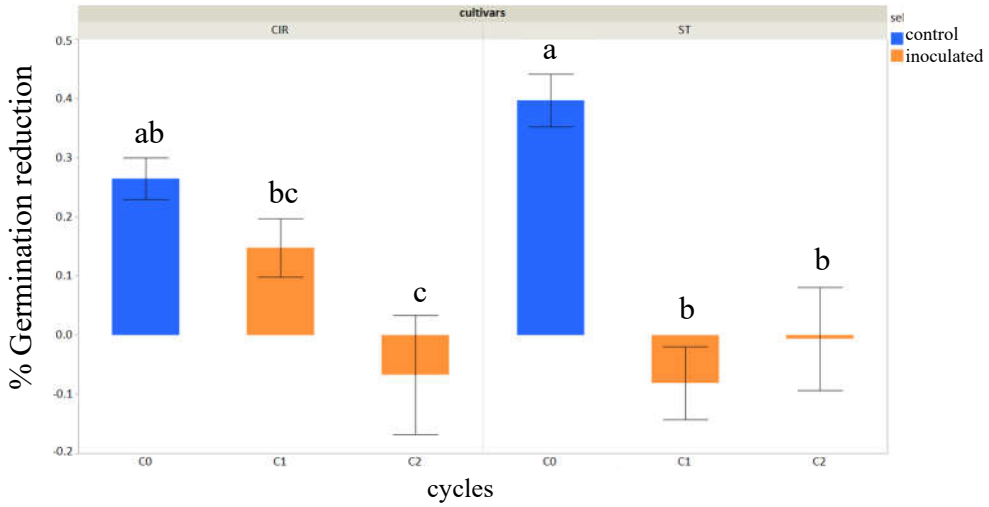
Table 3.4. Mean PLC (s.e.) and number of ST progenies from specific crosses of each cross type and reciprocal crosses (shaded).

| Cross type | Female | male | Mean PLC (s.e.) | Number progenies |
|---|---|---|---|---|
| RR | R10 | R6 | 28.65 (2.28) | 48 |
| RR | R6 | R10 | 28.54 (2.19) | 48 |
| RR | R11 | R2 | 36.56 (2.45) | 48 |
| RR | R2 | R11 | 38.54 (2.54) | 48 |
| RR | R14 | R4 | 28.96 (2.22)* | 48 |
| RR | R4 | R14 | 44.00 (3.82)* | 35 |
| RS | R10 | S8 | 25.52 (1.83) | 48 |
| SR | S8 | R10 | 35.42 (2.3) | 48 |
| RS | R2 | S10 | 28.72 (2.03) | 48 |
| SR | S10 | R2 | 36.77 (2.04) | 48 |
| RS | R2 | S2 | 36.67 (2.47) | 48 |
| SR | S2 | R2 | 30.94 (2.38) | 48 |
| RS | R3 | S9 | 39.15 (3.23) | 48 |
| SR | S9 | R3 | 37.92 (2.51) | 48 |
| RS | R7 | S5 | 41.20 (3.34) | 48 |
| SR | S5 | R7 | 35.00 (1.92) | 72 |
| SS | S10 | S5 | 35.94 (3.01) | 48 |
| SS | S5 | S10 | 34.58 (2.1) | 48 |
| RR | R7 | R1 | 5.00 (NA) | 1 |
| RS | R10 | S7 | 30.83 (2.78) | 36 |
| RS | R2 | S12 | 35.47 (2.9) | 43 |
| RS | R9 | S4 | 45.31 (2.99) | 48 |
| SR | S10 | R11 | 35.24 (1.92) | 48 |
| SR | S14 | R1 | 30.62 (2.28) | 48 |
| SR | S14 | R5 | 28.54 (2.07) | 48 |
| SR | S6 | R9 | 28.12 (2.63) | 48 |
| SS | S14 | S11 | 30.10 (2.14) | 48 |
| SS | S3 | S9 | 28.12 (2.51) | 47 |
| SS | S6 | S4 | 25.00 (2.22) | 48 |

* means significantly different between reciprocal crosses at $\alpha = 0.05$.

The difference in heritability estimates between both resistances reflected a different progress from selection. For resistance to BSR, the selection showed the improvement of the resistance via a decrease in reduction in germination in each cycle of selection from 0.27, 0.15 to -0.07 in cycle 0, 1, and 2 in CIR and 0.40, -0.08 to -

0.01 in cycle 0, 1, and 2 in ST (Figure 3.4). The significantly decreased reduction in germination was evident in cycle 2 in CIR and cycle 1 in ST. Additionally, the germination of inoculated seeds increased in each cycle from 0.44, 0.50, to 0.60 in cycle 0, 1, and 2 in CIR; however, the germination of inoculated seeds increased from 0.21 to 0.51 in cycle 0 to 1 but did not increase in cycle 2 at 0.46 in ST (Figure 3.5). In CIR, the difference between the control and inoculated germination percentages was significant in cycle 0 and 1, but the difference was not significant in cycle 2. Whereas, in ST, the difference was significant only in cycle 0 but not significant since cycle 1. Interestingly, although the germination percentages of inoculated seeds in both CIR and ST kept increasing in each cycle of selection, the germination percentages of the control seeds did not significantly increase in CIR and increased once in C1 but stopped increasing in cycle 2 in ST.




Different letters represent significantly different group in each cultivar from Tukey's HSD at $\alpha = 0.05$.

Figure 3.4. Progress of selection for BSR in %germination reduction from C0, C1 and C2 of CIR and ST.

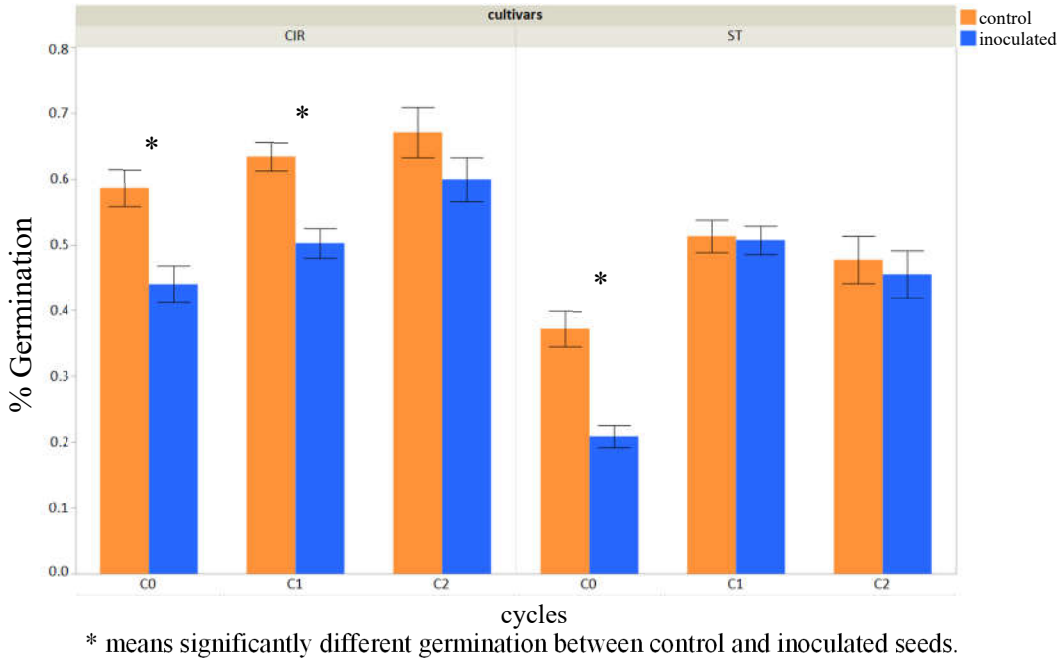



Figure 3.5. Progress from selection in %germination for resistance to BSR in CIR and ST comparing among C0, C1, and C2.

Such an improvement provided an impressive genetic gain from selection for BSR based on germination reduction percentage. In CIR, the genetic gains were 44 and 145% of cycle 0 to 1, and 1 to 2 or 95% per cycle (

Table 3.5). In ST, the genetic gains were 79 and 92% of cycle 0 to 1, and 1 to 2 or 86% per cycle. Based on these genetic gains, liability heritabilities ranged from 0.11 to 0.27 in CIR and -0.09 to 0.56 in ST.

Despite success in selection for resistance to BSR, the progress from selection for resistance to BLS was consistent with low or non-significant heritability. The selection did not show significant improvements in the resistance of CIR or ST (Figure 3.6). As such, the genetic gain and realized heritability were not significantly different from zero.

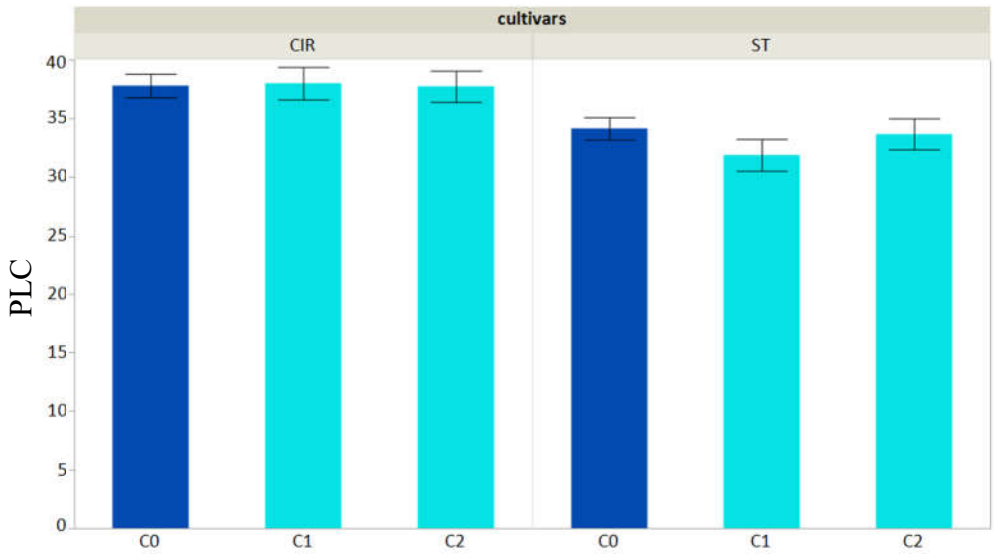
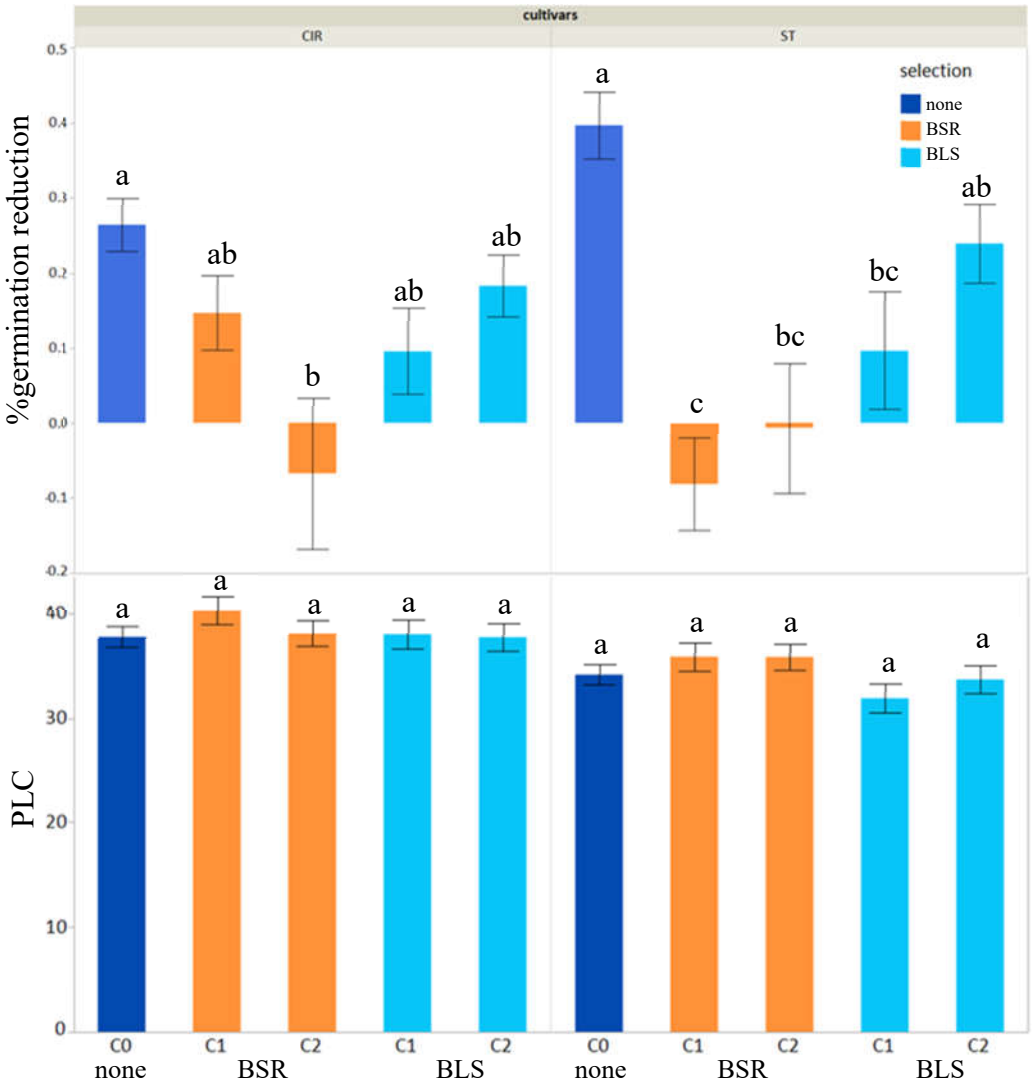



Figure 3.6 Progress from selection as in PLC for resistance to BLS in CIR and ST.

Table 3.5 Summary of selection for resistance to BSR and BLS: % gain, liability or realized heritability compared with theoretical individual-based narrow sense heritability from half-sib progenies.

| Cultivars | Trait | Comparisons | %Gain | Liability or realized heritability | Individual-based $h^2$ (s.e.) |
|---|---|---|---|---|---|
| CIR | BSR | C0 vs C1 | 44.53$^{ns}$ | 0.11$^{ns}$ | 0.33 (0.16) |
| | | C0 vs C2 | 125.32* | 0.22* | |
| | | C1 vs C2 | 145.64$^{ns}$ | 0.27$^{ns}$ | |
| | BLS | C0 vs C1 | -0.53$^{ns}$ | -0.01$^{ns}$ | 0.07 (0.08) |
| | | C0 vs C2 | 0.26$^{ns}$ | 0.00$^{ns}$ | |
| | | C1 vs C2 | 0.79$^{ns}$ | 0.01$^{ns}$ | |
| ST | BSR | C0 vs C1 | 79.55* | 0.56* | NA |
| | | C0 vs C2 | 101.60* | 0.32* | |
| | | C1 vs C2 | 92.17$^{ns}$ | -0.09$^{ns}$ | |
| | BLS | C0 vs C1 | 6.73$^{ns}$ | 0.08$^{ns}$ | NA |
| | | C0 vs C2 | 1.46$^{ns}$ | 0.01$^{ns}$ | |
| | | C1 vs C2 | -5.64$^{ns}$ | 0.05$^{ns}$ | |

After selection, the reciprocal test was conducted by testing resistance to BLS to the populations selected for BSR and testing resistance to BSR to the populations selected for BLS. The lesser reduction in germination was showed in the population selected for resistance to BSR as expected, but such a lesser reduction was also found in populations selected for BSR in C2 of both CIR and ST. (Figure 3.7). However, the percentage of germination reductions of CIR selected for BLS were not significantly different from the reductions of based population, but ST selected for BLS cycle 1 was significantly different than cycle 0, and cycle 2 was not. In contrast, none of selected ST and CIR from either for BLS and BRS showed progress in resistance to BLS in PLC. Due to the difference in improvement of selection between BSR and BLS, the genetic correlation between the resistances was not significantly different from zero (Table 3.6).




Different letters represent significantly different group in each cultivar from Tukey's

Figure 3.7 Comparison between progresses of selection for resistance to BSR in %germination reduction (orange) and BLS in PLC (light blue) in CIR (left) and ST (right).

Table 3.6 additive genetic correlations between BSR and BLS in CIR and ST.

| Cultivars | Comparisons | Correlations |
|---|---|---|
| CIR | C0 vs C1 | $17.89^{ns}$ |
| | C0 vs C2 | $-0.74^{ns}$ |
| | C1 vs C2 | $-4.29^{ns}$ |
| ST | C0 vs C1 | $-0.70^{ns}$ |
| | C0 vs C2 | $-1.24^{ns}$ |
| | C1 vs C2 | $0.08^{ns}$ |

**Discussion**

Although the heritability was higher in half-sib selection, individual selection was chosen to avoid inbreeding depression in later generations (Breese and Hayward, 1972). Each disease (BSR or BLS) resistance showed a different level of heritability. Moderate to high heritability of resistance to BSR resulted in high gain from selection for resistance to BSR whereas non-significant heritability of resistance to BLS confirmed no advance in selection for resistance for BSR. Such difference between the traits can be explained in that the mechanisms of resistance to the same pathogen were different. It was similar in barley infected by *Bipolaris sorokiniana* (Sacc.) Shoemaker, causing root rot and spot blotch and common beans infected with *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*Xcp*) on leaves, pods, and seeds (Arnaud-Santana et al., 1994; Kutcher et al., 1994). However, brown spot and root rot caused by *Pleiochaeta setosa* (Kircbn.) in narrow-leafed lupin (*Lupinus angustifolius* L.) showed significant correlation between the resistance (Cowling et al., 1997).

The progress of selection for BSR resistance was very high. Even within one cycle of selection, the gains from selection were 44 and 79% in CIR and ST, respectively. However, such gains were comprised of the effects of the germination capacity itself and not solely resistance to BSR. The selected seeds for this resistance

must germinate and be resistant to the disease. The selection cannot screen for the resistant seed without germination. As a result, the germination of uninoculated seeds increased along with each cycle of selection in ST but not in CIR (Figure 3.5).

For resistance to Bipolaris leaf spot, normally distributed segregation from test crosses suggested that the trait was controlled by many alleles with small effects since the R-R or R-S progenies were more resistant than S-S progenies in both CIR and ST. However, in an actual selection and bigger sample, the low and non-significant heritability of the trait predicted no progress of selection. This could be explained by many hypotheses. First, without natural pressure of the disease, the resistant allele frequency was possibly low so the recurrent phenotypic selection needed more cycles to increase the frequency to show resistance improvement (Hallauer and Darrah, 1985; Labate et al., 1997; Kolawole et al., 2017). Second, the major defense to the fungus could potentially occur in seeds because the primary inoculation occurred on seed, and the transmission rate from seed to seedling can reach to 80% (Barnwal et al., 2013). Such a high natural pressure in seed disease was consistent with high heritability of the resistance to BSR. Lastly, there was variation in inoculation among trays, as indicated by the variation of the susceptible check among trays. Since this selection has been conducted on only two cultivars based on local adaptation, the future breeding program should expand to screen more populations for the resistance.

After a progressive selection for resistance to BSR, the next step for that population will be a yield trial before possibly releasing the cultivar for use commercially. Moreover, the selection method for BSR can be simply integrated to other switchgrass breeding programs via seed inoculation before other specific traits.

Another lesson from these selections was the lack of improvement of resistance to BLS. The low or non-significant heritability, normally distributed segregation from test crosses, and no correlation to resistance to BSR provide an opportunity to improve breeding separately from BSR. Bigger populations can be screened to explore resistance that could be conferred by many alleles. To verify these resistant alleles in such a complicated switchgrass population, genomic data should be utilized for marker-assistant or genomic selection in the future.

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# CHAPTER 4

## GENOME-WIDE ASSOCIATIONS WITH RESISTANCE TO BIPOLARIS LEAF SPOT IN A NORTHERN SWITCHGRASS POPULATION


### Abstract

Switchgrass (*Panicum virgatum* L.), a northern native perennial grass, suffers from yield reduction from Bipolaris leaf spot (BLS) caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker. To determine the candidate resistant populations via multiple phenotyping approaches and identify potential resistance genes, single- and multi-trait genome-wide association studies (GWAS) were conducted in the northern switchgrass population. The resistance to BLS was evaluated from both natural and artificial inoculations. The natural inoculation from the field was evaluated in two locations – NY and PA, and artificial inoculations by leaf detachment and leaf disk assay were conducted and evaluated via both vision and image analysis. The most resistant population based on a combination of leaf detachment via image analysis (DTIA)-leaf disk via image analysis (DSIA)-mean from two locations was 'SW805' population. There were only four reliable GWAS results from different trait combinations in different subsets of genotypes including 1) full set of 479 genotypes with DTIA-DSIA-highest score from NY (MNY), 2) tetraploid with MNY, 3) lowland with DTIA-DSIA-MNY and highest score from PA (MPA), and 4) upland with leaf detachment via vision (DTVI)-MNY. None of the four GWAS provided significant overlapping regions. Overall, the resistance genes were associated with 18 markers on chromosomes 1b, 2a, 2b, 3a, 3b, 5a, 5b, 6a, 7a, 7b, 8a, 9a, and 9b and accumulatively explained phenotypic variance of DSIA by 26.72% at the most and DTIA by 6.32% at

the least. Within linkage disequilibrium of 20 kb, the potential resistance genes included genes encoding Myb, cytochrome P450, isocitrate lyase, E3 ubiquitin-protein ligase, mitogen-activated kinases, glutathione S-transferase, Birefringence-like 32, ABC transporter, etc.

## Introduction

Switchgrass (*Panicum virgatum* L.) is a perennial biomass crop native to North America. Biomass and other agronomic traits have been the focus on improvement. Although many diseases have been reported to cause deleterious effects on yield, research on disease resistance, especially breeding for the resistance, is scarce. *Bipolaris oryzae* (Breda de Haan) Shoemake (BO) is one of the major fungi causing Bipolaris leaf spot (BLS) in switchgrass that can reduce biomass by 70% (Fajolu, 2012). The approach for the disease management was to breed for improvement of resistance to BLS.

The natural distribution of switchgrass is broad latitudes across eastern side of the Rocky Mountain. Simple morphological differentiation of switchgrass can separate into two groups of upland and lowland ecotypes. Upland ecotypes are well adapted to higher latitudes and higher drought tolerance whereas lowland ecotypes provide higher yield and require more water (Stroup et al., 2003). The more in-depth genetic diversity was revealed by Network-Based single nucleotide polymorphism (SNP) discovery protocol suggesting the differentiation of switchgrass group by isolation-by-ploidy, the migration from south to north, and incidence of tetraploid upland from octaploid upland (Lu et al., 2013). Such a large diversity within switchgrass populations can provide a source of resistance.

Since BO is a necrotrophic pathogen, the interaction between switchgrass and the fungus was modelled as an inverse gene-for-gene model (Friesen et al., 2007). In general, the pathogen secretes necrotrophic effectors as host-selective toxins (HST) that interact with host sensitivity genes, resulting in a compatible susceptible

interaction to trigger host cell death (Friesen et al., 2008). Such an interaction is known as effector-triggered susceptibility (ETS). Interestingly, according to comparative genome study (Condon et al., 2013), BO does not produce any HST. Regardless of HST, BO produces ophiobolin A and B as a non-host-selective toxin (Xue et al., 2015) triggering many groups of proteins and enzymes such as reactive oxygen species (ROS) detoxification, protein phosphorylation, and ethylene production (Kim et al., 2014; Bockhaven et al., 2015).

Such an ETS interaction naturally removes the individuals with the necrotrophic effectors from the population. Breeding for the improvement of resistance to BLS can be done by eliminating susceptible genes from the population. However, the recurrent phenotypic selection for the resistance in 'Shelter' and 'Cave-in-Rock' for two cycles of selection did not provide any improvement (Chapter 3). Breeding for the resistance by various approaches, stages, and populations was suggested. Therefore, identification of resistant populations is a crucial step.

To accelerate breeding for disease resistance, genomics-assisted breeding is an important approach. Basically, it begins with gene identification, isolation, cloning, functional characterization, validation and utilization. There are two approaches to identify resistance genes: linkage mapping and genome-wide association studies (GWAS). Although GWAS cannot confirm the causal polymorphism, GWAS depends on linkage disequilibrium (LD) between the markers and the causal polymorphisms. With high allelic diversity and ancestral recombination events in the diversity panel, GWAS yields a finer resolution than linkage mapping (Yu and Buckler, 2006). The technique has been used to dissect flowering time in switchgrass (Grabowski et al.,

2017). Although GWAS has never been used to dissect disease resistance in switchgrass, it has successfully dissected resistance to leaf rust caused by *Puccinia triticina* Eriks., tan spot caused by *Pyrenophora tritici-repentis* (Die.) Shoemaker and stripe rust caused by *Puccinia striiformis* West. in wheat (*Triticum aestivum* L.) (Juliana et al., 2018). Despite no resistance genes identified in switchgrass, there were 13 SNP markers from quantitative trait loci (QTLs) linked with the resistance in rice (Sato et al., 2008, 2015). This study will provide the first dissection of the resistance to BLS in switchgrass.

The objectives were 1) to determine genotypes or populations from the northern switchgrass association panel that can be candidates for resistance to BLS, 2) to conduct a single- and multi-trait GWAS for resistance to BLS in the association panel via both natural and artificial inoculation, and 3) to explore the genes linked to the significant markers from the reliable GWAS model to determine potential candidate genes for resistance to BLS.

## Materials and Methods

### Switchgrass in the Northern Association Panel

The switchgrass population used in this study has been developed for accelerating breeding progress especially for bioenergy traits at northern latitude (Lu et al., 2013; Lipka et al., 2014). The population consists of 479 genotypes from 66 populations representing the mostly upland northern population and some southern lowland population. It was initially planted in Ithaca, NY, in 2008 and then vegetatively cloned and planted in a randomized, complete block design with three

replicates in 0.9 m spaced planting in Ithaca, NY, and Philipsburg, PA, in 2016 (Figure 4.1). No fungicide was applied to the field.


Figure 4.1. Switchgrass Northern Association Panel of 66 populations and 479 genotypes with 3 clones in each location in Ithaca, NY (upper) and Phillipsburg, PA (lower) in 0.9-m spaced plot.

**Disease evaluation and phenotype processing**

Bipolaris leaf spot has been evaluated in both fields to determine the resistance in natural incidence and laboratory to minimize environmental effects on the resistance. Field evaluation was conducted in the same week in both locations in August 2017. Each plant was visually evaluated, the severity ranging from 0 to 5 as 0

= 0% BLS, 1= 1-10% BLS, 2= 11-25% BLS, 3= 26-50% 134 BLS, 4= 51-75% BLS, and 5= 76-100% of the leaf area with BLS (Figure 4.2). With artificial inoculation in the laboratory, disease severity was evaluated by leaf detachment and leaf disk assay by taking leaf samples from one replicate of genotypes in Ithaca, NY. In the leaf detachment assay, three healthy leaves were randomly selected from 30 centimeters below the top of the shoot to control the same stage of leaf development. The leaves were cut into two inches, washed with deionized water, placed in a pre-wet petri dish bedded with filter paper, kept cold in a cooler, and refrigerated overnight.

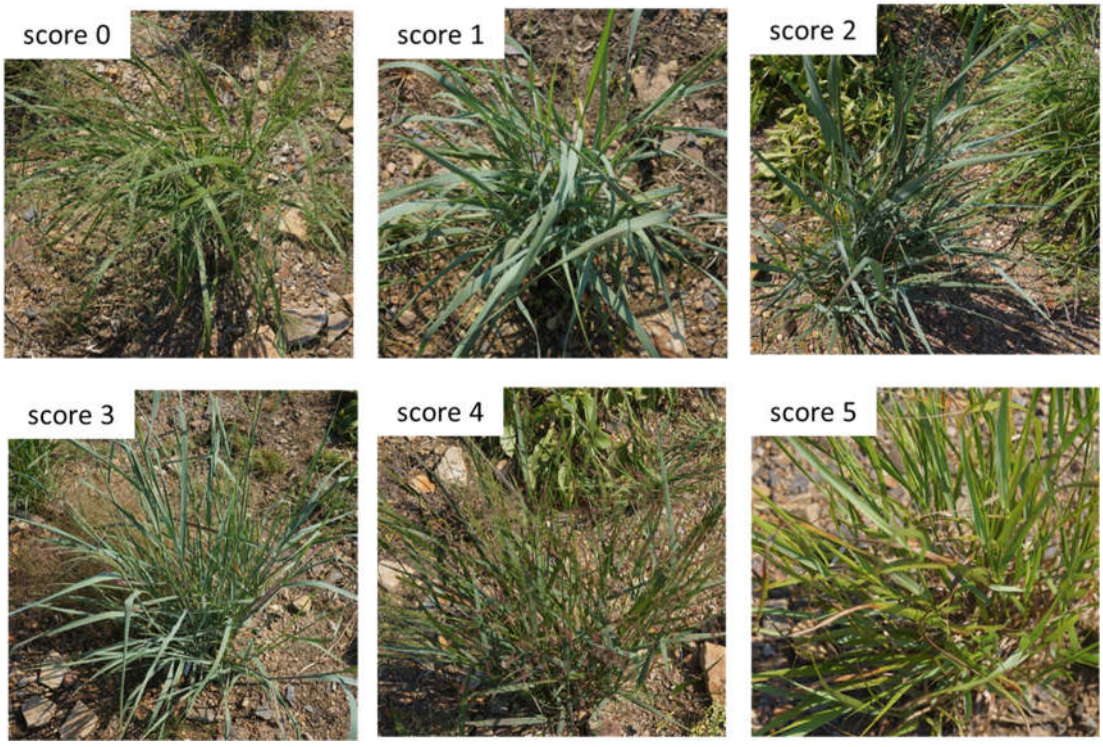


Figure 4.2. Rating of field evaluation ranges from 0 = 0% BLS, 1= 1-10% BLS, 2= 11-25% BLS, 3= 26-50% BLS, 4= 51-75% BLS, 5= 76-100% BLS.

The inoculum was prepared from a subculture of a single conidium isolated from a ‘Carthage’ switchgrass leaf in the warm season biofuels field experiment in Ithaca, NY, by Katie Waxman. The day after collecting the detached leaf, the three-

week-old plate was flooded, filtered via gauze, and the concentration adjusted to $10^5$ conidia/ml by hemocytometer with 2 drops of Tween-20 per 100 ml. An airbrush pressuring at 10 psi was used to spray inoculum on each dry-surfaced adaxial side of the leaf in each plate. After letting the droplets of inoculum dry and firmly attach to the leaf surface, the plates were then sealed with a paraffin tape to maintain high moisture condition. The plates were kept at room temperature with 24-hour light for seven days (Figure 4.3). The disease evaluation was conducted by vision and image analysis software. Both evaluations were based on percentage of leaf covered with lesions. The evaluation by vision was from 0 to 100 % with 10% increment. Then each leaf was dried and scan with a flatbed scanner (Canon CanoScan LiDE 700F) at a resolution of 1,200 dpi, and images were saved in .tiff format. Due to the limitation of laboratory space for the plates and labor in leaf collection, 85 to 200 genotypes were sampled in each set of the experiment, with some overlapping genotypes in 1 to 3 duplicated plates and additional non-inoculated plates. The leaf samplings were done weekly from May to June 2017. A total of seven sets of experiment eventually covered all of 482 genotypes. Such an unbalanced design was handled with best linear unbiased prediction (BLUP) to extract random effect of the genotype by fitting the experiment factor as a fixed effect.

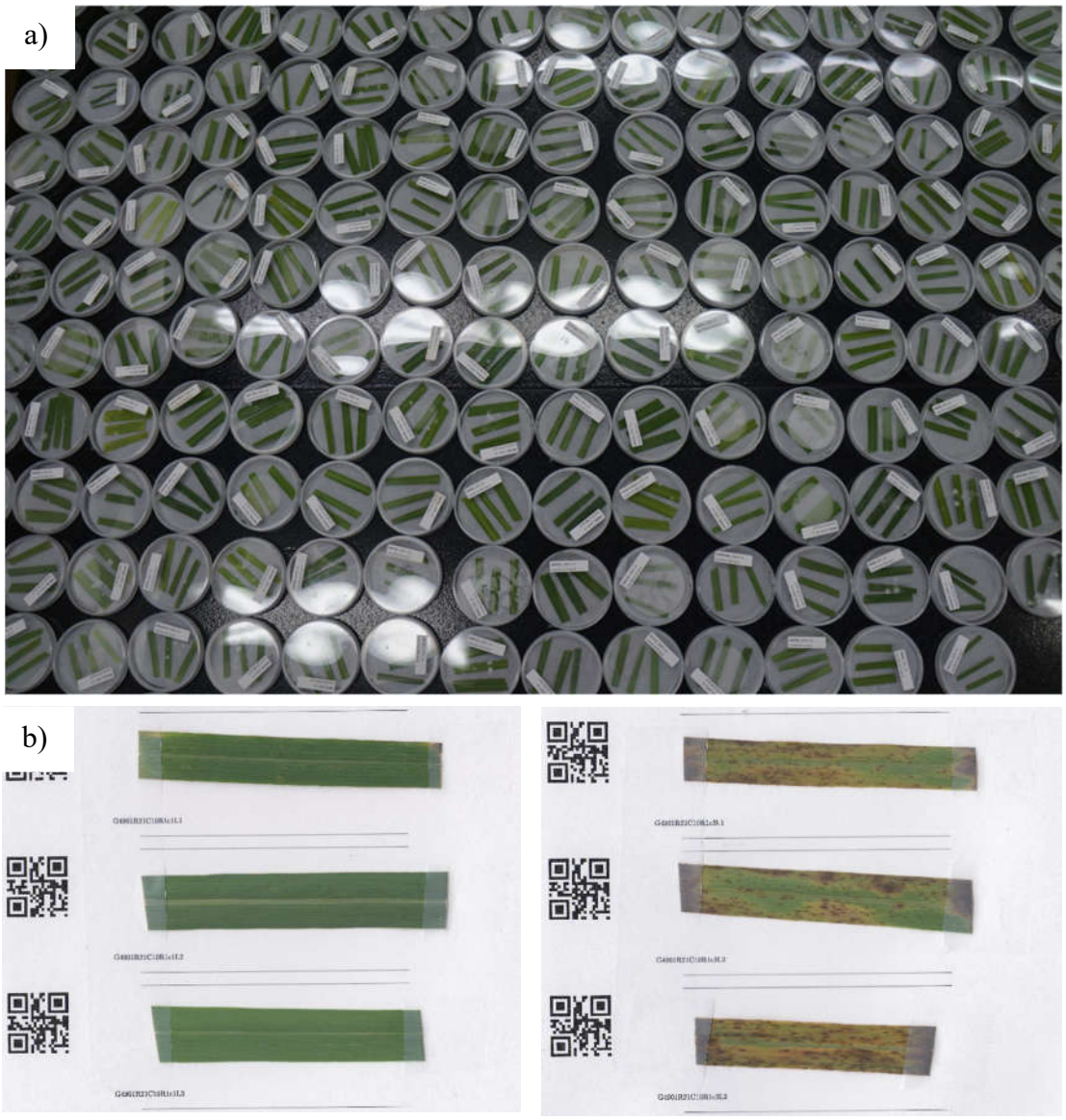


Figure 4.3. Leaf detachment assay; a) sealed plates of detached leaves under 24-h light 25°C; b) control inoculated (left) and inoculated leaves (right) at 7 dpi were taped on white paper with QR code label for scanning for image analysis.

In the leaf disk evaluation, all of 479 genotypes were collected at the same day in August 2017 by cutting the same age leaves from each genotype and keeping them refrigerated overnight. On the next day, the leaves were washed with deionized water, the surface dried, cut by keeping the midrib in the middle into an 8-mm disk, and

placed on a water agar plate. There were 28 leaf disks in each plate, including four check disks (control and inoculated disks of resistant 'SW43_09' and susceptible 'SW122_02') and three replicated disks of eight genotypes (Figure 4.4). The inoculum was prepared the same way described above. For each leaf, two µL of inoculum was dropped in the middle of the right side of the leaf. After letting the droplet dry, the plates were sealed with paraffin tape to keep high moisture and kept under 12-hour light at room temperature. The disease evaluation was conducted at seven dpi both by vision and image analysis software. The percent of leaf disk covered with lesions and necrotic tissue was assessed by a vision from 0 to 100 with ten increments. Also, on the same day, a photo was taken each plate with a digital DSLR Cannon Rebel T6 DSLR camera with the resolution of 2644 x 3084 in .JPG.

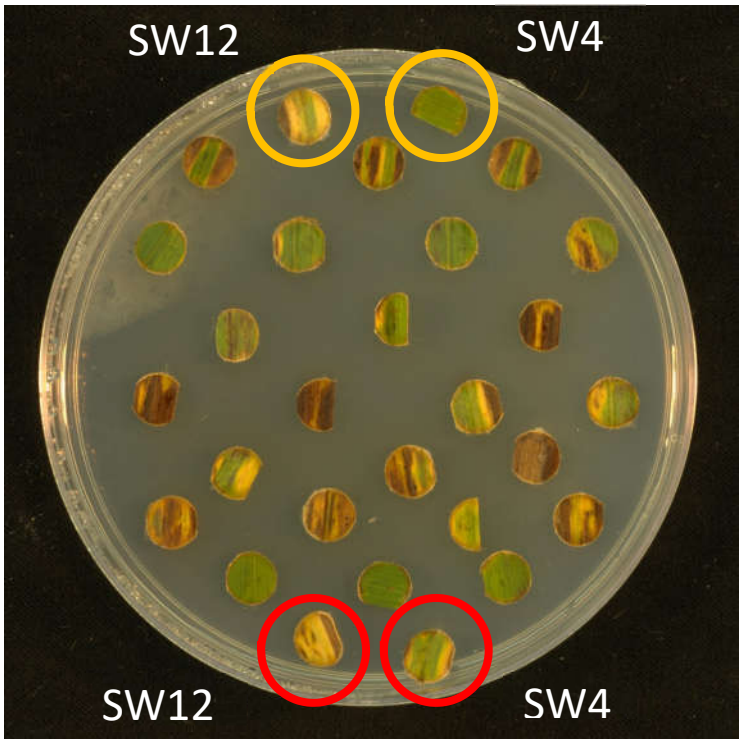


Figure 4.4. A plate example of leaf disk assay at 7 dpi. Each plate consisted of 28 leaf disks. SW43_09 was used as a resistant check and SW122_02 as a susceptible check. Control leaf disks (yellow circles) and inoculated leaf disks (red circles) were included in all plates.

Images from both leaf detachment and leaf disk assays were analyzed by using ImageJ macro at the setting provided in the appendix. Each detached leaf or leaf disk was measured for total leaf area and total chlorosis lesion area (yellow and black area). Percentage of area covered with lesions was computed by dividing the total necrotic lesion area by total leaf area.

Package 'LME4' (Bates et al., 2015) in R was used to calculate Best Linear Unbiased Prediction (BLUPs) from various disease evaluations. For the field evaluation, BLUPs were computed based on each location separately and on the two locations combined using genotypes and replicates as random effects in each location model. BLUPs for the two locations combined were fitted genotype, replicates, locations, the interaction between genotypes and location, as random effects. Besides generating BLUPs from field evaluation, the highest score, which is most severe symptoms observed from each location and two locations over replicates, were also used due to successful QTLs of resistance to foliar symptoms caused by potato virus Y in autotetraploid potato (*Solanum tuberosum* L.) (Silva et al., 2017). For disease evaluations under laboratory condition, since all phenotypes were in percentages to leaf area, log transformation was performed before computing BLUPs. For the leaf detachment assay, BLUPs were computed with experiment, genotype, the interaction between genotypes and experiment, plates within the experiment, and leaf replicates of genotypes as random effects. For the leaf disk assay, BLUPs were calculated with plates as a fixed effect and genotype, replicates within plates, and genotype within plates as random effects. Broad-sense heritability was estimated from variances in each model. Moreover, phenotypic correlations were computed between resistance to

Bipolaris leaf spot to the other 20 morphological and biomass quality traits from Lipka et al. (2014), such as plant height, anthesis date, acid detergent lignin, minerals, ethanol/g dry weight, etc.

Therefore, the resistance to BLS was evaluated via three approaches – leaf detachment, leaf disk, and field evaluation – providing phenotyped traits including BLUPs of detached leaf percent lesion via vision and image analysis (DTVI and DTIA), BLUPs of leaf disk percent lesion via vision and image analysis (DSVI and DSIA), BLUPs of field evaluation from two locations, NY and PA (BTL, BNY, and BPA), highest score from two locations, NY and PA (MTL, MNY, and MPA).

**Genotyping, linkage disequilibrium analysis, and population structure**

HAPMAP v.1 set from Evans et al. (2015) provided 1,377,841 single-nucleotide polymorphisms covering 38,654 genic regions. In short, DNA of each genotype was processed via exome-capture using the Roch-Nimblegen switchgrass exome-capture probe set (Evans et al., 2014) and DNA sequencing. The sequences were aligned to the *P. virgatum* genome assembly v.1.1 (*P. virgatum* v.1.1, DOE-JGI, http://phytozome.jgi.doe.gov/) for SNP discovery. In this calling, SNPs were required to be bi-allelic, sequenced in all samples, be monomorphic in at least two samples, and have > 5X coverage in > 95% of the samples. At each SNP, the genotype dosages ranged from zero to two copies of minor alleles and can be nonintegers by using EM algorithm (Martin et al., 2010). Naturally, the switchgrass association panel includes allopolyploid switchgrass: tetraploids (4x), octoploids (8x) and hexaploids (6x). It is challenging to perform GWAS with polyploid models. In this study, we modelled them under the assumption of disomic inheritance similar to the study of GWAS of

flowering time in Grabowski et al. (2017). This was because tetraploid switchgrass was confirmed to show disomic inheritance (Okada et al., 2010). Although octaploid switchgrass had four copies of each homologous chromosome, which was difficult to precisely determine heterozygous genotypes, the disomic segregation was used for the model with the caution of the increasing standard error of estimates in GWAS. Moreover, linkage disequilibrium (LD) was computed via '-r2' tag in PLINK as $r^2$ between all SNPs within 1 MB and recorded all values (Purcell et al., 2007). Principle component analysis was used to evaluate the population structure via TASSEL v.5 (Trait Analysis by aSSociation Evolution and Linkage) (Bradbury et al., 2007)

**Genome-wide association studies**

Genome-wide Efficient Mixed Model Association (GEMMA) (Zhou and Stephens, 2012) was used to implement a multivariate linear mixed model for GWAS of single- and multiple-phenotyping approaches for resistance to BLS. Kinship was included as a random effect. Also, the first three principal components (PCs) were included as fixed covariates. Although the results of Bayesian information criterion resulted in no improvement of the GWAS model with PC inclusion (Lipka et al., 2013), the inclusion of PCs helped to improve the QQ-plot closer to the theoretical QQ-plot for this resistance to BLS. The GWAS was conducted in five groups including all of 479, lowland, upland, tetraploid (4X), and octaploid (8X) genotypes to determine if there were any SNPs linked to specific switchgrass population. Minor allele frequency (MAF) at 0.05 was used to filter the minor alleles. To correct multiple testing, false discovery rate (FDR) was used for a cut-off value at 0.1. The significant markers then used to determine candidate genes linked to them within the range of LD

and search in JBrowse in *P. virgatum* v.1.1 in Phytozome (e.g., if the position of the marker was 77345950 on chromosome 2a and LD was 20 kb, "Chr02a:77335950..77355950" was used). In case that the candidate gene was not fully aligned within that LD range, we considered the gene as a candidate if 80% of its length was within the LD range. Since some of SNPs' positions could not be aligned to the *P. virgatum* genome assembly v.1.1, they were grouped as U1-U4 chromosome and were excluded from determining the candidate genes.

**Candidate genes based on resistance in rice**

Since *B. oryzae* is one of the major pathogens in rice (*Oryza sativa*), major resistant QTLs have been identified in recombinant inbred lines (RILs) and double haploids (DH) by using restriction fragment length polymorphism (RFLP), simple-sequence repeat (SSR) markers, and sequence- tagged site (STS) (Sato et al., 2008, 2015; Katara et al., 2010). Three major QTLs in Chromosomes 1, 4 and 11 were then studied further in near-isogenic lines (NILs) and 13 SNP markers linked with the resistance (Sato et al., 2015). The NILs are BC3F5 between indica 'Tadukan' (resistance) and temperate japonica 'Koshihikari' (susceptible). According to GWAS in rice, linkage disequilibrium (LD) are ~ 100 kb in indica and ~200 kb temperate japonica (Zhao et al., 2011). Thus, in this study, the candidate resistance genes were screened from 200 kb around the 13 SNPs from *Oryza sativa* genome assembly v.7.0 (*Oryza sativa* v.7.0, DOE-JGI, http://phytozome.jgi.doe.gov/). The candidate genes from rice are listed in the Appendix. The sequences of these candidate genes were used to identify potential homologs in switchgrass by using the BLASTP tool on Phytozome (Goodstein et al., 2012).

## Results

### Phenotyping and correlation between traits

The results from leaf detachment showed that 'ECS.6' was the most resistant population (DTVI 24% and DTIA 8%) and 'SW115' was the most susceptible population (DTVI 81% and DTIA 60%) (Table 4.1). However, when comparing each genotype, 'SW788.05' was the most resistant genotype (DTVI 6% and DTIA 2%) whereas 'SW63.05' was the most susceptible genotype (Appendix). The result from the leaf disk assay showed that 'SW803' was the most resistant population (DSVI 20% and DSIA 48%) and 'SW38' was the most susceptible population (DSVI 98% and DSIA 99%). In the genotype-based comparison, 'High Tide.02' showed the most resistance (DSVI 10% and DSIA 11%), but the most susceptible were 81 genotypes with DSVI 100% and DSIA 100%. In the field evaluation based on the mean of the severity score (0 to 5) from two locations, 'Shelter' appeared to be the most resistant population (0.44) and 'SW787' was the most susceptible. However, when considering each location, different populations performed differently due to high GxE effect. In NY, SW803 showed the most resistant population (0.22) and SW787 appeared to be the most susceptible one (3.0). Whereas, in PA, Shelter appeared to be the most resistant one (0.33) and Pathfinder appeared to be the most susceptible population (2.9). As Silva et al. (2017) phenotyped potato tuber necrotic ringspot disease by using highest score for the disease evaluation across replicates to be able to review significant QTLs, the highest score for BLS was considered to be used in this study also. In two locations, 'SW123', 'SW33', 'SW793', 'SW781', High Tide, and Timber had the lowest MTL at 3 (resistant), and 41 populations had the highest MTL at 5

(susceptible). In NY, SW123, SW128 and 'ECS.6' had the lowest MNY at 2, and 32 populations had the highest MNY at 5. In PA, 'SW115', 'SW802', 'SW31' and 'SW43' had the lowest MPA at 2, and 19 populations had the highest MPA at 5. The most resistant and susceptible genotype in each phenotyping approach is in the Appendix. Based on artificial inoculations, both the leaf detachment and the leaf disk assay suggested that the lowland ecotypes (DTVI 43%, DTIA 21%, DSVI 43% and DSIA 70%) appeared to be more resistant than upland ecotypes (DTVI 61%, DTIA 39%, DSVI 67% and DSIA 89%); however, the field evaluation of means between two locations did not show the same trend (Figure 4.5).

Although each trait from different phenotyping approaches had significantly moderate to high broad-sense heritability (H) (Table 4.2), $H^2$ of severity from two locations was zero. This was suggested by the different trends of the mean of severity from two locations, NY and PA (Figure 4.6). Due to zero heritability, the BTL was zero and cannot be used for conducting GWAS. The various trends were not only present in the field evaluation, various phenotyping approaches yielded various results of resistance as suggested by the various trend of DTIA, DSIA and mean from two locations and correlation among the traits (Figure 4.6 and Figure 4.7). For example, from 479 genotypes, 'KY1625_07' ranked 27 from DTIA but ranked 260 in DSIA, and 100 in mean from two locations. Such differences led to low correlation among approaches such that the $r^2$ between DTIA and DSIA was only 0.1 and between DTIA and mean of two locations was 0.03 (Figure 4.7). Besides most low correlations, there were high correlations in DTVI-DTIA ($r^2 = 0.84$) and DSVI-DSIA ($r^2 = 0.85$). There

was no BLS resistance trait correlating to other agronomic or biomass quality traist (Figure 4.8).

Table 4.1 Phenotype summary of severity of Bipolaris leaf spot from mean of Bipolaris lesion percentage from leaf detachment assay by vision (DTVI), by image analysis (DTIA), the severity from the leaf disk assay by vision (DSVI), by image analysis (DSIA), mean of two locations, highest scores between two locations (MTL), mean in NY, highest score in NY (MNY), mean in PA, and highest score in PA (MPA) based on population (rank).

| Population | Ecotype | DTVI | DTIA | DSVI | DSIA | Mean two fields | MTL | Mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.6 | Lowland North | 0.24 (1) | 0.08 (1) | 0.76 (53) | 0.94 (51) | 1.5 (53) | 4 (7) | 0.5 (6) | 2 (1) | 2 (64) | 4 (27) |
| SW805 | Lowland North | 0.29 (2) | 0.1 (2) | 0.27 (5) | 0.56 (4) | 0.78 (9) | 4 (7) | 1 (27) | 4 (15) | 0.67 (12) | 4 (27) |
| SW803 | Lowland North | 0.31 (3) | 0.12 (3) | 0.2 (1) | 0.48 (2) | 1.11 (25) | 5 (25) | 0.22 (1) | 3 (4) | 2.11 (65) | 5 (48) |
| High.Tide | Lowland North | 0.33 (4) | 0.13 (4) | 0.55 (22) | 0.72 (15) | 1.22 (32) | 3 (1) | 1 (27) | 3 (4) | 1.22 (38) | 3 (26) |
| SW795 | Lowland North | 0.36 (5) | 0.14 (5) | 0.49 (19) | 0.75 (18) | 1 (24) | 5 (25) | 0.62 (8) | 5 (35) | 1 (33) | 3 (26) |
| Shelter | Upland East | 0.37 (6) | 0.15 (6) | 0.6 (32) | 0.92 (47) | 0.44 (1) | 4 (7) | 0.78 (14) | 4 (15) | 0.33 (3) | 4 (27) |
| SW799 | Lowland North | 0.38 (1) | 0.16 (1) | 0.68 (46) | 0.86 (32) | 0.67 (1) | 4 (7) | 1 (27) | 4 (15) | 0.33 (3) | 3 (26) |
| SW797 | Lowland North | 0.39 (9) | 0.21 (16) | 0.44 (15) | 0.72 (15) | 1 (24) | 4 (7) | 1 (27) | 3 (4) | 1 (33) | 4 (27) |
| SW798 | Lowland North | 0.39 (9) | 0.17 (9) | 0.31 (6) | 0.61 (6) | 1.2 (31) | 5 (25) | 1.6 (47) | 5 (35) | 1 (33) | 3 (26) |
| SW781 | Lowland North | 0.4 (10) | 0.24 (18) | 0.33 (1) | 0.68 (10) | 1 (24) | 3 (1) | 1 (27) | 3 (4) | 1.17 (37) | 3 (26) |
| SW782 | Upland East | 0.42 (11) | 0.19 (11) | 0.63 (35) | 0.84 (29) | 0.6 (4) | 4 (7) | 0.8 (17) | 4 (15) | 0.6 (10) | 4 (27) |
| SW806 | Lowland North | 0.43 (12) | 0.2 (14) | 0.41 (13) | 0.69 (11) | 1.44 (49) | 5 (25) | 1.67 (50) | 4 (15) | 1.89 (58) | 5 (48) |
| SW43 | Upland North | 0.44 (14) | 0.18 (10) | 0.48 (18) | 0.76 (19) | 1.43 (48) | 5 (25) | 2.14 (58) | 5 (35) | 0.57 (9) | 2 (1) |
| SW802 | Lowland North | 0.44 (14) | 0.2 (14) | 0.37 (10) | 0.63 (8) | 0.6 (4) | 4 (7) | 0.8 (17) | 4 (15) | 0.8 (21) | 2 (1) |

| Population | Ecotype | DTVI | DTIA | DSVI | DSIA | Mean two fields | MTL | Mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.1 | Lowland North | 0.45 (16) | 0.26 (24) | 0.38 (11) | 0.63 (8) | 1.2 (31) | 5 (25) | 0.4 (3) | 4 (15) | 1.6 (52) | 5 (48) |
| KY1625 | Upland East | 0.45 (16) | 0.17 (9) | 0.46 (17) | 0.82 (25) | 1.8 (59) | 5 (25) | 1.2 (33) | 5 (35) | 1.9 (59) | 4 (27) |
| SW38 | Upland West | 0.46 (19) | 0.35 (35) | 0.98 (66) | 0.99 (64) | 1 (24) | 5 (25) | 1 (27) | 5 (35) | 1 (33) | 3 (26) |
| SW788 | Lowland North | 0.46 (19) | 0.21 (16) | 0.26 (4) | 0.54 (3) | 1 (24) | 5 (25) | 0.78 (14) | 3 (4) | 1.56 (48) | 5 (48) |
| SW790 | Lowland/Upland Mix | 0.46 (19) | 0.29 (26) | 0.72 (50) | 0.89 (38) | 1 (24) | 4 (7) | 1.5 (42) | 4 (15) | 0.75 (16) | 3 (26) |
| ECS.11 | Upland East | 0.48 (21) | 0.2 (14) | 0.66 (41) | 0.81 (24) | 1.2 (31) | 4 (7) | 1.6 (47) | 3 (4) | 1 (33) | 4 (27) |
| SWG39 | Lowland South | 0.48 (21) | 0.22 (17) | 0.73 (51) | 0.92 (47) | 1.83 (61) | 4 (7) | 1.5 (42) | 4 (15) | 1.67 (53) | 4 (27) |
| Carthage | Upland West | 0.5 (23) | 0.25 (20) | 0.87 (61) | 0.99 (64) | 1.83 (61) | 5 (25) | 1.67 (50) | 5 (35) | 2 (64) | 4 (27) |
| SW793 | Lowland North | 0.5 (23) | 0.25 (20) | 0.25 (3) | 0.48 (2) | 0.8 (12) | 3 (1) | 0.4 (3) | 3 (4) | 1 (33) | 3 (26) |
| SW127 | Upland West | 0.51 (25) | 0.39 (44) | 0.66 (41) | 0.88 (36) | 1.5 (53) | 5 (25) | 1.12 (30) | 5 (35) | 1.88 (56) | 5 (48) |
| SWG32 | Lowland South | 0.51 (25) | 0.26 (24) | 0.56 (24) | 0.83 (26) | 1.86 (63) | 5 (25) | 2.57 (65) | 5 (35) | 1.14 (36) | 4 (27) |
| SW110 | Upland West | 0.52 (27) | 0.39 (44) | 0.66 (41) | 0.92 (47) | 1.5 (53) | 4 (7) | 1.3 (36) | 4 (15) | 2 (64) | 4 (27) |
| SW796 | Lowland North | 0.52 (27) | 0.26 (24) | 0.58 (29) | 0.84 (29) | 0.75 (8) | 4 (7) | 0.75 (12) | 3 (4) | 0.62 (11) | 4 (27) |
| SW65 | Upland East | 0.53 (29) | 0.3 (27) | 0.71 (49) | 0.86 (32) | 1.25 (33) | 5 (25) | 1.5 (42) | 5 (35) | 1.12 (35) | 3 (26) |
| SW789 | Lowland/Upland Mix | 0.53 (29) | 0.26 (24) | 0.21 (2) | 0.74 (16) | 1.4 (46) | 4 (7) | 1.8 (52) | 4 (15) | 1.6 (52) | 3 (26) |
| WS98.SB | Upland Mix | 0.54 (30) | 0.33 (31) | 0.68 (46) | 0.86 (32) | 0.88 (13) | 4 (7) | 0.5 (6) | 4 (15) | 1.25 (39) | 3 (26) |
| ECS.10 | Upland East | 0.55 | 0.41 | 0.37 | 0.58 | 1.33 | 5 | 1.33 | 5 | 1 | 3 |

| Population | Ecotype | DTVI | DTIA | DSVI | DSIA | Mean two fields | MTL | Mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (33) | (48) | (10) | (5) | (40) | (25) | (38) | (35) | (33) | (26) |
| ECS.12 | Upland West | 0.55 (33) | 0.32 (28) | 0.8 (57) | 0.97 (58) | 1.43 (48) | 5 (25) | 0.86 (19) | 4 (15) | 1.43 (45) | 5 (48) |
| Timber | Lowland South | 0.55 (33) | 0.33 (31) | 0.4 (12) | 0.75 (18) | 1.57 (54) | 3 (1) | 1.57 (44) | 3 (4) | 1.29 (42) | 3 (26) |
| Dacotah | Upland North | 0.56 (34) | 0.38 (41) | 0.46 (17) | 0.78 (22) | 1.67 (57) | 5 (25) | 2.5 (64) | 5 (35) | 0.83 (24) | 3 (26) |
| Blackwell | Upland West | 0.57 (36) | 0.33 (31) | 0.58 (29) | 0.87 (33) | 1.4 (46) | 5 (25) | 1.3 (36) | 4 (15) | 1.7 (54) | 5 (48) |
| SW123 | Upland East | 0.57 (36) | 0.27 (25) | 0.68 (46) | 0.93 (48) | 0.67 (1) | 3 (1) | 0.67 (10) | 2 (1) | 0.78 (17) | 3 (26) |
| SW33 | Upland West | 0.58 (37) | 0.35 (35) | 0.34 (8) | 0.64 (9) | 0.8 (12) | 3 (1) | 1.2 (33) | 3 (4) | 0.8 (21) | 3 (26) |
| SW40 | Upland North | 0.59 (38) | 0.34 (32) | 0.56 (24) | 0.92 (47) | 1.67 (57) | 5 (25) | 2 (56) | 5 (35) | 0.83 (24) | 5 (48) |
| Cave.in.Rock | Upland East | 0.6 (41) | 0.37 (38) | 0.64 (37) | 0.95 (55) | 1.4 (46) | 5 (25) | 1.1 (29) | 5 (35) | 1.6 (52) | 5 (48) |
| Kanlow | Lowland South | 0.6 (41) | 0.38 (41) | 0.53 (21) | 0.78 (22) | 1.4 (46) | 4 (7) | 1.1 (29) | 4 (15) | 1.4 (44) | 4 (27) |
| SW63 | Upland North | 0.6 (41) | 0.42 (52) | 0.88 (62) | 0.99 (64) | 0.5 (2) | 5 (25) | 0.67 (10) | 5 (35) | 0.83 (24) | 4 (27) |
| SW808 | Upland East | 0.61 (42) | 0.38 (41) | 0.66 (41) | 0.89 (38) | 1 (24) | 5 (25) | 0.86 (19) | 5 (35) | 0.86 (25) | 5 (48) |
| SW128 | Upland West | 0.62 (45) | 0.4 (46) | 0.62 (34) | 0.94 (51) | 0.67 (1) | 3 (1) | 0.44 (4) | 2 (1) | 1.33 (43) | 3 (26) |
| SW46 | Upland North | 0.62 (45) | 0.41 (48) | 0.83 (59) | 0.95 (55) | 1.33 (40) | 5 (25) | 2 (56) | 5 (35) | 0.89 (26) | 3 (26) |
| WS4U | Upland North | 0.62 (45) | 0.44 (55) | 0.9 (63) | 0.99 (64) | 1.5 (53) | 5 (25) | 2.5 (64) | 5 (35) | 0.5 (8) | 4 (27) |
| SW114 | Upland North | 0.63 (48) | 0.36 (36) | 0.67 (43) | 0.92 (47) | 1.86 (63) | 5 (25) | 2.29 (60) | 5 (35) | 1.29 (42) | 5 (48) |
| SW49 | Upland North | 0.63 (48) | 0.4 (46) | 0.74 (52) | 0.95 (55) | 1.29 (34) | 5 (25) | 2.14 (58) | 5 (35) | 0.5 (8) | 5 (48) |

| Population | Ecotype | DTVI | DTIA | DSVI | DSIA | Mean two fields | MTL | Mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW787 | Upland North | 0.63 (48) | 0.35 (35) | 0.43 (14) | 0.78 (22) | 2.33 (66) | 5 (25) | 3 (66) | 5 (35) | 1.44 (46) | 5 (48) |
| SW109 | Upland West | 0.65 (50) | 0.42 (52) | 0.81 (58) | 0.98 (59) | 1.89 (64) | 5 (25) | 1.56 (43) | 5 (35) | 1.89 (58) | 5 (48) |
| SW116 | Upland North | 0.65 (50) | 0.37 (38) | 0.78 (56) | 0.97 (58) | 1.38 (41) | 5 (25) | 2.5 (64) | 5 (35) | 0.38 (4) | 3 (26) |
| SW58 | Upland West | 0.66 (53) | 0.42 (52) | 0.71 (49) | 0.92 (47) | 0.89 (14) | 5 (25) | 0.56 (1) | 5 (35) | 1.11 (34) | 5 (48) |
| SW64 | Upland East | 0.66 (53) | 0.56 (64) | 0.58 (29) | 0.72 (15) | 0.8 (12) | 5 (25) | 1.3 (36) | 5 (35) | 0.7 (14) | 4 (27) |
| SW809 | Upland East | 0.66 (53) | 0.46 (58) | 0.59 (31) | 0.91 (40) | 1.33 (40) | 4 (7) | 1.33 (38) | 4 (15) | 2 (64) | 4 (27) |
| SW112 | Upland West | 0.67 (55) | 0.42 (52) | 0.62 (34) | 0.88 (36) | 1.3 (35) | 5 (25) | 0.7 (11) | 5 (35) | 1.5 (47) | 4 (27) |
| SW50 | Upland West | 0.67 (55) | 0.44 (55) | 0.92 (64) | 1 (66) | 1 (24) | 5 (25) | 0.8 (17) | 5 (35) | 1.6 (52) | 4 (27) |
| SW122 | Upland West | 0.68 (57) | 0.39 (44) | 0.57 (25) | 0.94 (51) | 2 (65) | 5 (25) | 1.67 (50) | 4 (15) | 1.83 (55) | 5 (48) |
| SW786 | Upland North | 0.68 (57) | 0.43 (53) | 0.77 (55) | 0.92 (47) | 1.14 (27) | 5 (25) | 1.86 (53) | 5 (35) | 0.71 (15) | 3 (26) |
| Pathfinder | Upland West | 0.69 (59) | 0.5 (60) | 0.67 (43) | 0.95 (55) | 1.8 (59) | 5 (25) | 1 (27) | 3 (4) | 2.9 (66) | 5 (48) |
| SW31 | Upland North | 0.69 (59) | 0.52 (62) | 0.77 (55) | 0.99 (64) | 1.33 (40) | 5 (25) | 2.17 (59) | 5 (35) | 0.33 (3) | 2 (1) |
| SW124 | Upland North | 0.7 (60) | 0.46 (58) | 0.64 (37) | 0.79 (23) | 1.6 (55) | 5 (25) | 2.3 (61) | 5 (35) | 0.7 (14) | 5 (48) |
| SW129 | Upland North | 0.72 (61) | 0.49 (59) | 0.58 (29) | 0.84 (29) | 1.2 (31) | 5 (25) | 1.7 (51) | 5 (35) | 0.5 (8) | 3 (26) |
| ECS.2 | Upland West | 0.74 (63) | 0.45 (56) | 0.59 (31) | 0.9 (39) | 1 (24) | 5 (25) | 1.4 (39) | 5 (35) | 0.8 (21) | 3 (26) |
| SW51 | Upland West | 0.74 (63) | 0.54 (63) | 0.97 (65) | 1 (66) | 1.14 (27) | 4 (7) | 1.14 (31) | 4 (15) | 1.29 (42) | 4 (27) |
| Sunburst | Upland West | 0.76 | 0.57 | 0.86 | 0.96 | 1.33 | 5 | 1 | 4 | 2 | 5 |

| Population | Ecotype | DTVI | DTIA | DSVI | DSIA | Mean two fields | MTL | Mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (65) | (65) | (60) | (56) | (40) | (25) | (27) | (15) | (64) | (48) |
| SW102 | Upland North | 0.76 (65) | 0.52 (62) | 0.69 (47) | 0.88 (36) | 1.4 (46) | 5 (25) | 1.9 (54) | 5 (35) | 0.8 (21) | 4 (27) |
| SW115 | Upland North | 0.81 (66) | 0.6 (66) | 0.5 (20) | 0.71 (12) | 1 (24) | 4 (7) | 1.6 (47) | 4 (15) | 0.4 (5) | 2 (1) |

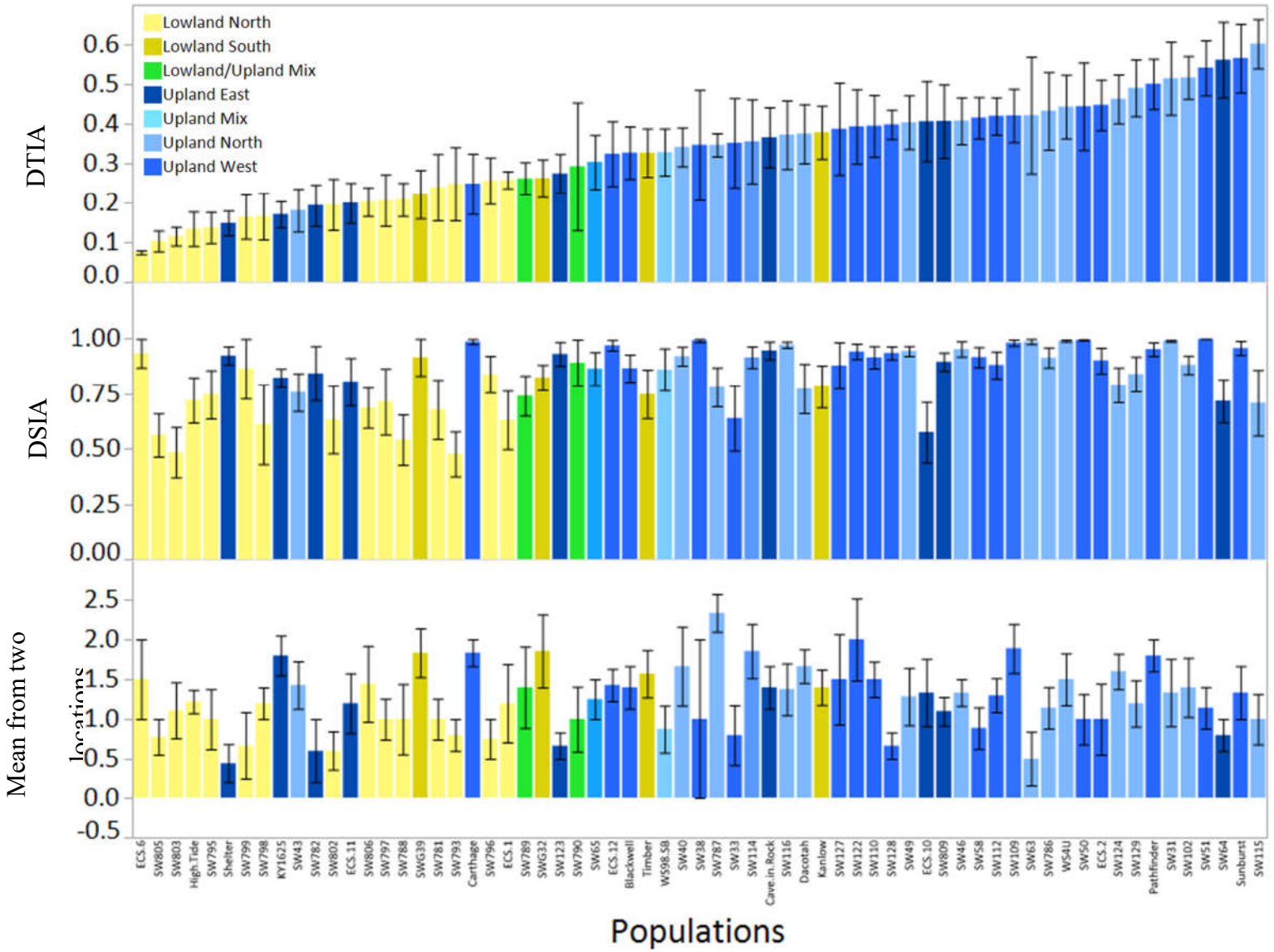


Figure 4.5. Histogram of mean severity from the leaf detachment assay via image analysis (DTIA) (top), from the leaf disk assay via image analysis (DSIA) (middle) and mean field severity from two locations with standard error from each of 66 populations. They were ordered based on DTIA and colored based on ecotypes.

Table 4.2. Broad-sense heritability (s.e.) of each trait.

| **Traits** | **H (s.e.)** |
|---|---|
| Severity from the leaf detachment via vision (DTVI) | 0.76 ± 0.02 |
| Severity from the leaf detachment via image analysis (DTIA) | 0.74 ± 0.06 |
| Severity from the leaf disk assay via vision (DSVI) | 0.56 ± 0.05 |
| Severity from the leaf disk assay via image analysis (DSIA) | 0.54 ± 0.04 |
| BLUPs two locations | 0.003 ± 0.06 |
| BLUPs NY | 0.32 ± 0.05 |
| BLUPs PA | 0.61 ± 0.04 |

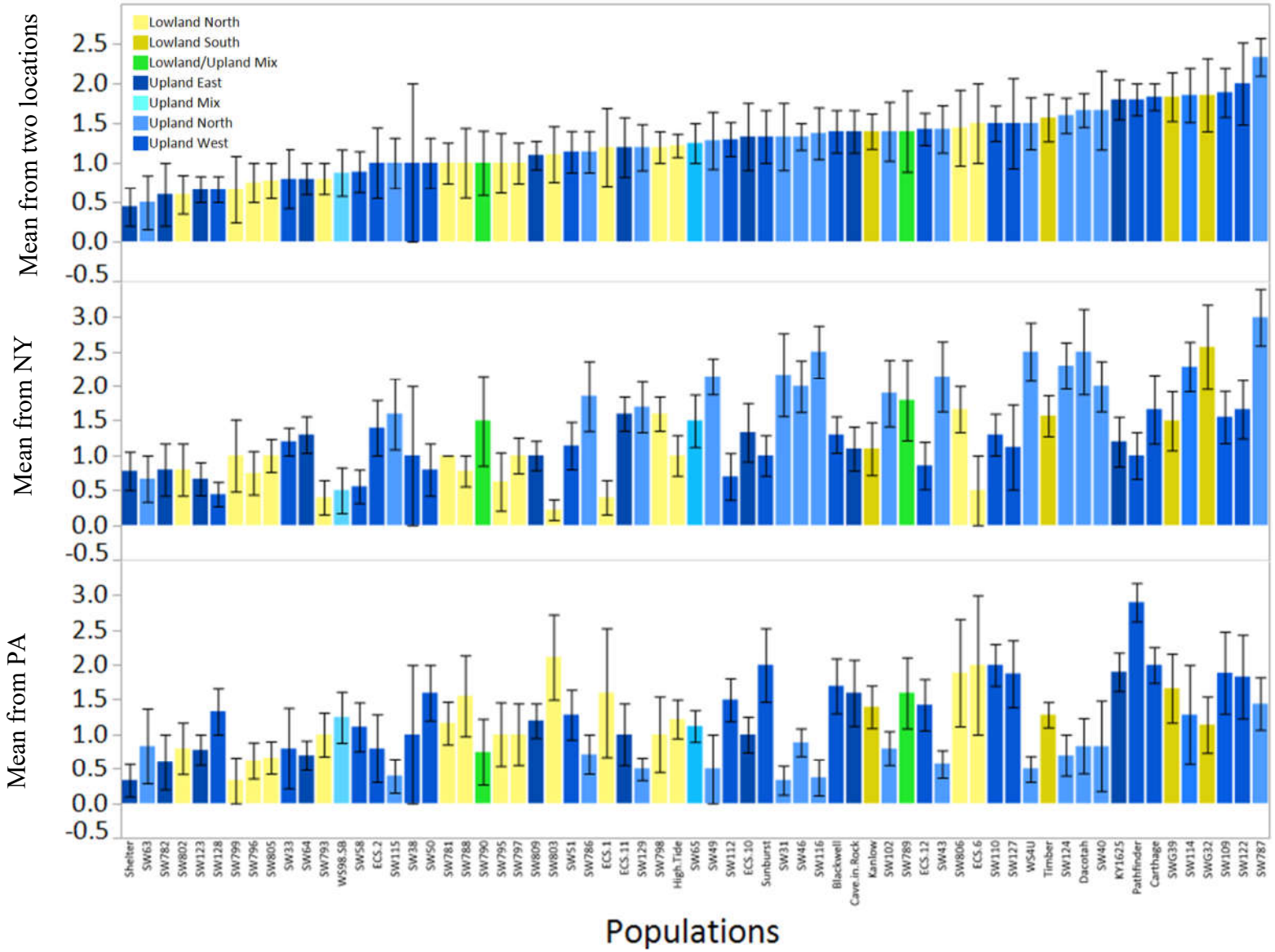


Figure 4.6. Histogram of mean severity score from 0 to 5 from two location (top), mean from NY (middle) and mean from PA with standard error from each 66 population. They were ordered based on mean from two locations and colored based on ecotypes.

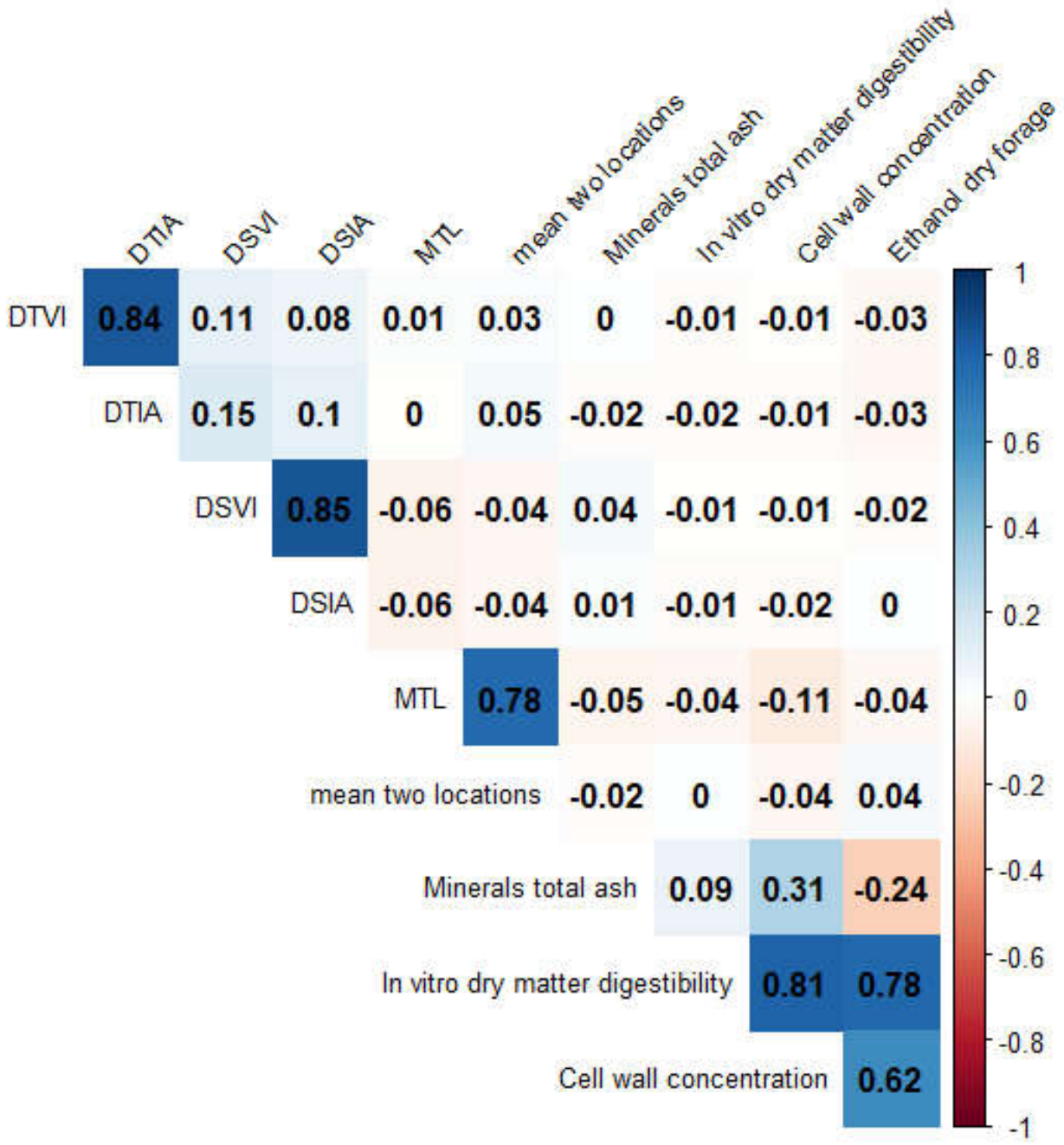


Figure 4.7. Correlation plot among BLUPs of severity from the leaf detachment via vison (DTVI), via image analysis (DTIA), from leaf disk assay via vision (DSVI), via image analysis (DSIA), the highest score between two locations (MTL), mean from two location, minerals total ash, In vitro dry matter digestibility, and cell wall concentration.

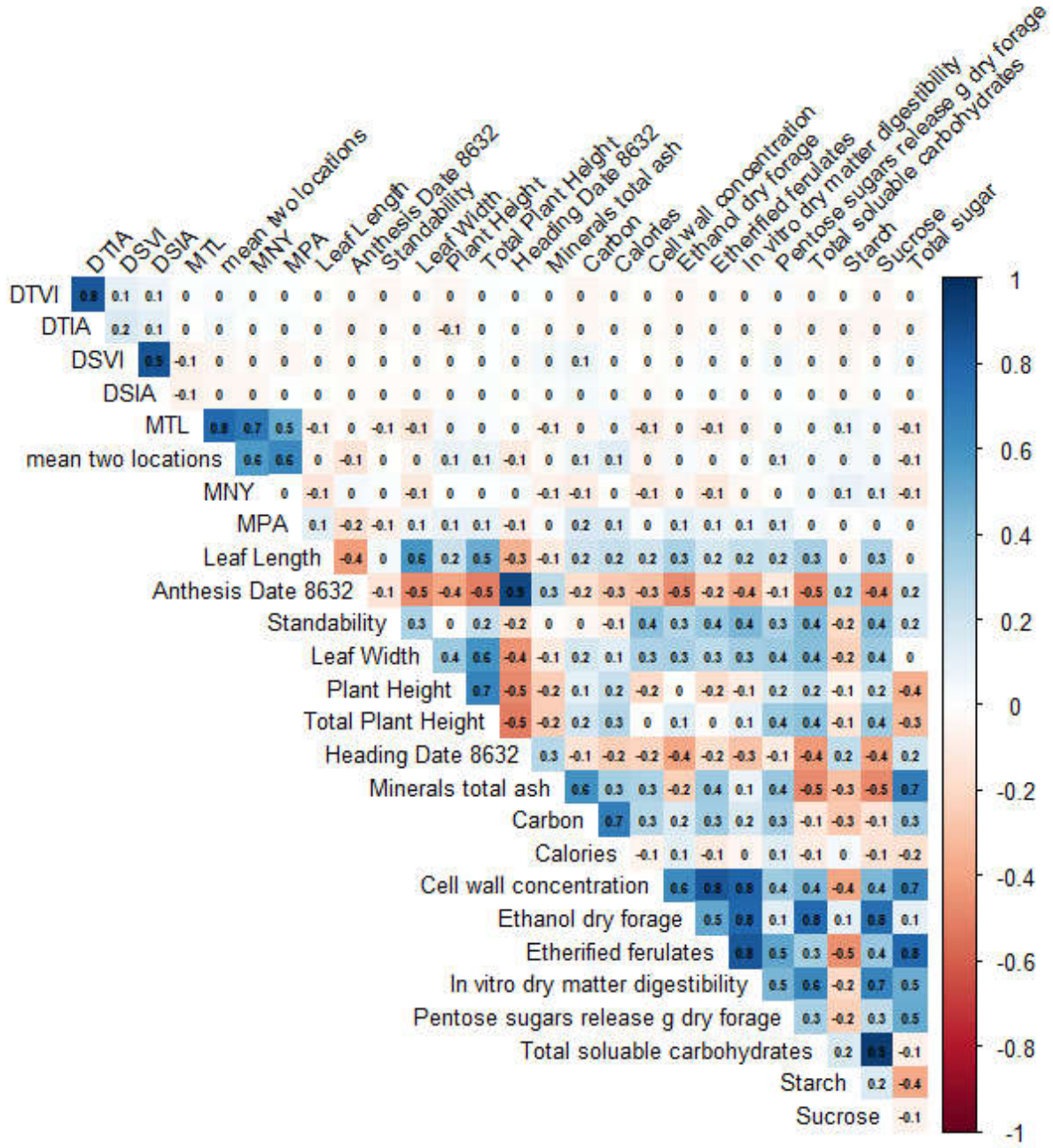


Figure 4.8. Correlation plot among BLUPs of severity from the leaf detachment via vision (DTVI), via image analysis (DTIA), from leaf disk assay via vision (DSVI), via image analysis (DSIA), the highest score between two locations (MTL), mean from two location, highest score in NY (MNY), highest score in PA (MPA) and BLUPs of other agronomic and biomass quality traits.

**Linkage disequilibrium and principal component analysis**

To determine the gene interval that potentially linked to the significant markers from GWAS, linkage disequilibrium (LD) decay was estimated by plotting the allele frequency correlations ($R^2$) against the physical distance in base pairs (Figure 4.9). In the full set of 479 genotypes, the LD decayed sharply within 20 kb and reached a plateau background within 50 kb. The other groups of 4X, 8X, lowland and upland genotypes showed a similar pattern of the LD despite being slightly different in an 8X group. Therefore, in this study, we focused on genes within the 20 kb from the significant SNPs.

Based on the principal component analysis, there was a strong population structure in this association panel (Figure 4.10). From PC1 and PC2, upland and lowland ecotypes were apart from each other. Also, within the lowland ecotype, the latitude of lowland can be distinguished to lowland north and south groups. The separation of upland ecotype can also be noticed in PC1 and PC3 in that the upland north was grouped apart from the other upland. Besides ecotypes, ploidy level can be differentiated into groups. Both lowland north and south, and upland north genotypes were tetraploid (4X) while the upland east and upland west genotypes were octaploid (8X). In total, PC1, PC2, and PC3 explained the variance due to the population of 15.03%.

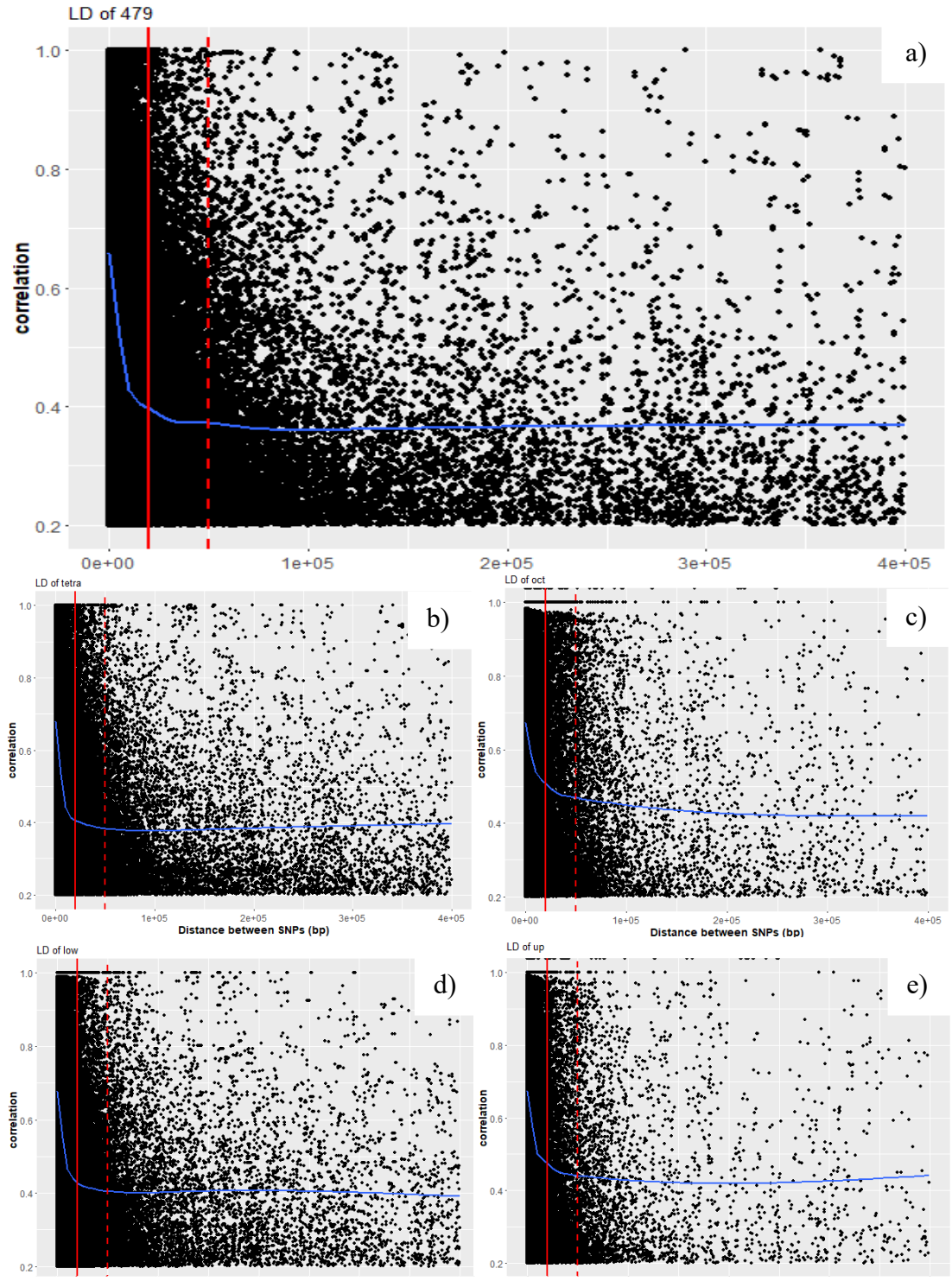


Figure 4.9. Scatter plot showing the linkage disequilibrium (LD) decay by plotting physical distance in base pairs against the LD estimate as correlation ($R^2$) in a) 479 genotypes, b) 4X group, c) 8X group, d) lowland ecotype, and e) upland ecotype. From full set of 479 genotypes, the LD (blue curve) decays rapidly within 20 kb (red line) and reaches background levels around 50 kb (dashed red line).

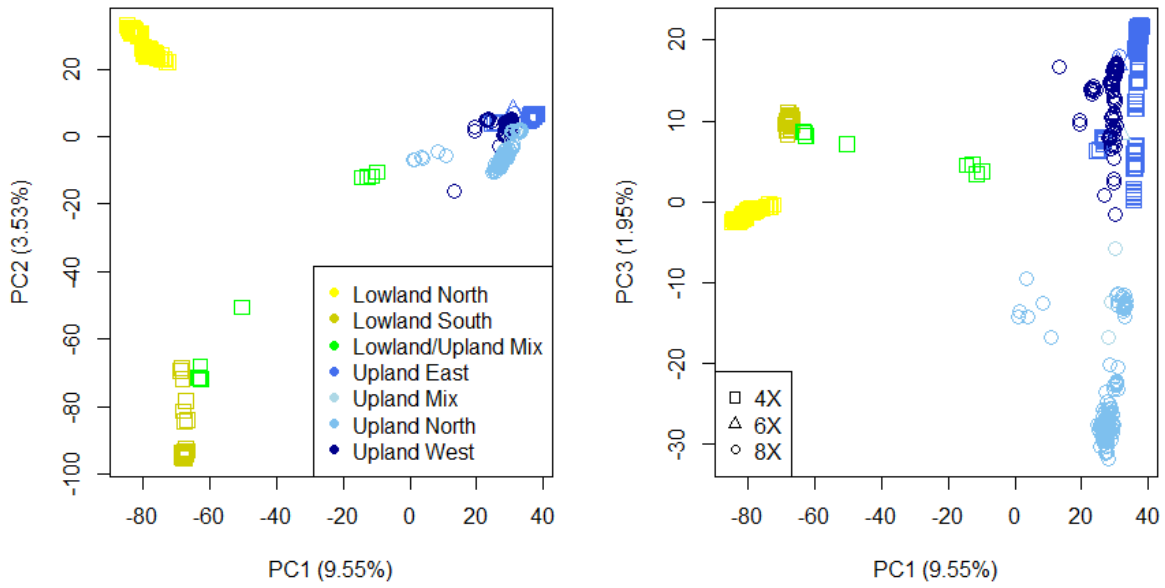


Figure 4.10. The 479 switchgrass genotypes from the Northern Association Panel showing the different distributions based on ecotype and region from principal component analysis (PC). The lowland ecotype can be differentiated into two regions from PC1 and PC2, and the upland ecotype can be grouped into three regions from PC1 and PC3.

**Genome-wide association mapping**

As we conducted GWAS with five subsets of genotypes including 479 upland, lowland, 4X and 8X to multiple combinations of assessing resistance to BLS via many phenotyping approaches, almost all the combinations for GWAS did not provide reliable results based on flat or inflated QQ-plots. In the full set of 479 genotypes, single traits from artificial inoculation via either leaf detachment or leaf disk assay with vision or image analysis (DTVI, DTIA, DSVI, and DSIA) provided reliable GWAS results based on inflated QQ-plot (Figure 4.11). Although DSIA showed the best QQ-plot (Figure 4.11d), the convex head and inflated tail of -log(P-value) suggested confounding of the association analysis. The single-trait GWAS of field evaluations (MTL, MNY, MPA, BNY, BPA, mean of two locations, mean of NY, and mean of PA) did not yield any reliable association analysis (Figure 4.12). The combination of two traits from artificial inoculations yielded better QQ-plots, but they still showed unreliable QQ-plots with a convex head and early inflation in the middle of the plot. Among the two-trait GWAS, DTIA-DSIA analysis provided the better GWAS with the QQ-plot that delayed the inflated tail from -log(P-value) of 2 to about 2.5. To improve the association analysis, the GWASs were then conducted with the combination of two artificial inoculations (DTIA-DSIA) with field evaluations. The only three-trait combination that provided reliable GWAS was DTIA-DSIA-MNY due to the QQ-plot with the expected distribution (Figure 4.13d).

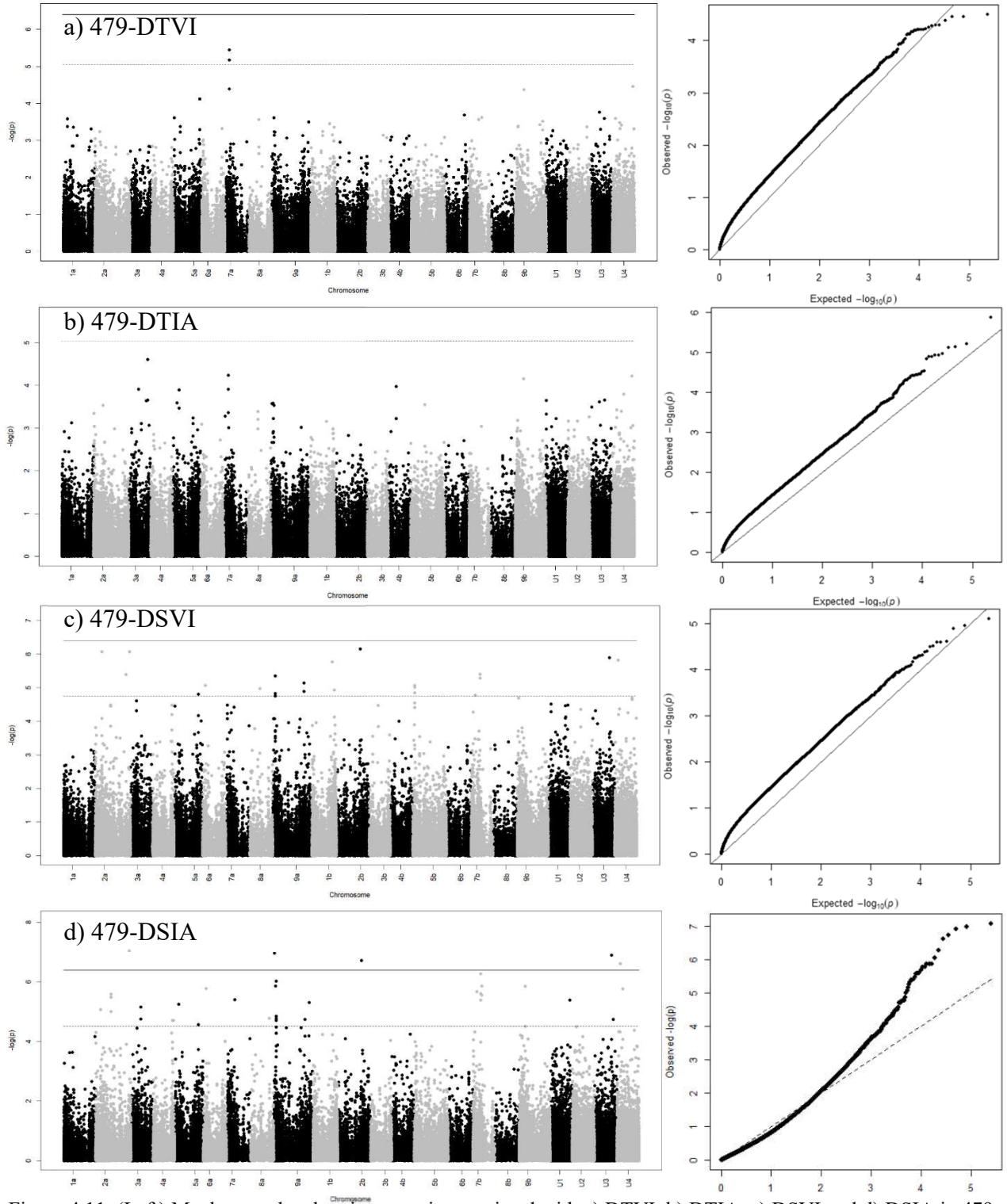


Figure 4.11. (Left) Manhattan plot showing genetic associated with a) DTVI, b) DTIA, c) DSVI and d) DSIA in 479 genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in 479 genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via vision (DSVI), and BLUPs of severity from the leaf disk assay via image analysis (DSIA).

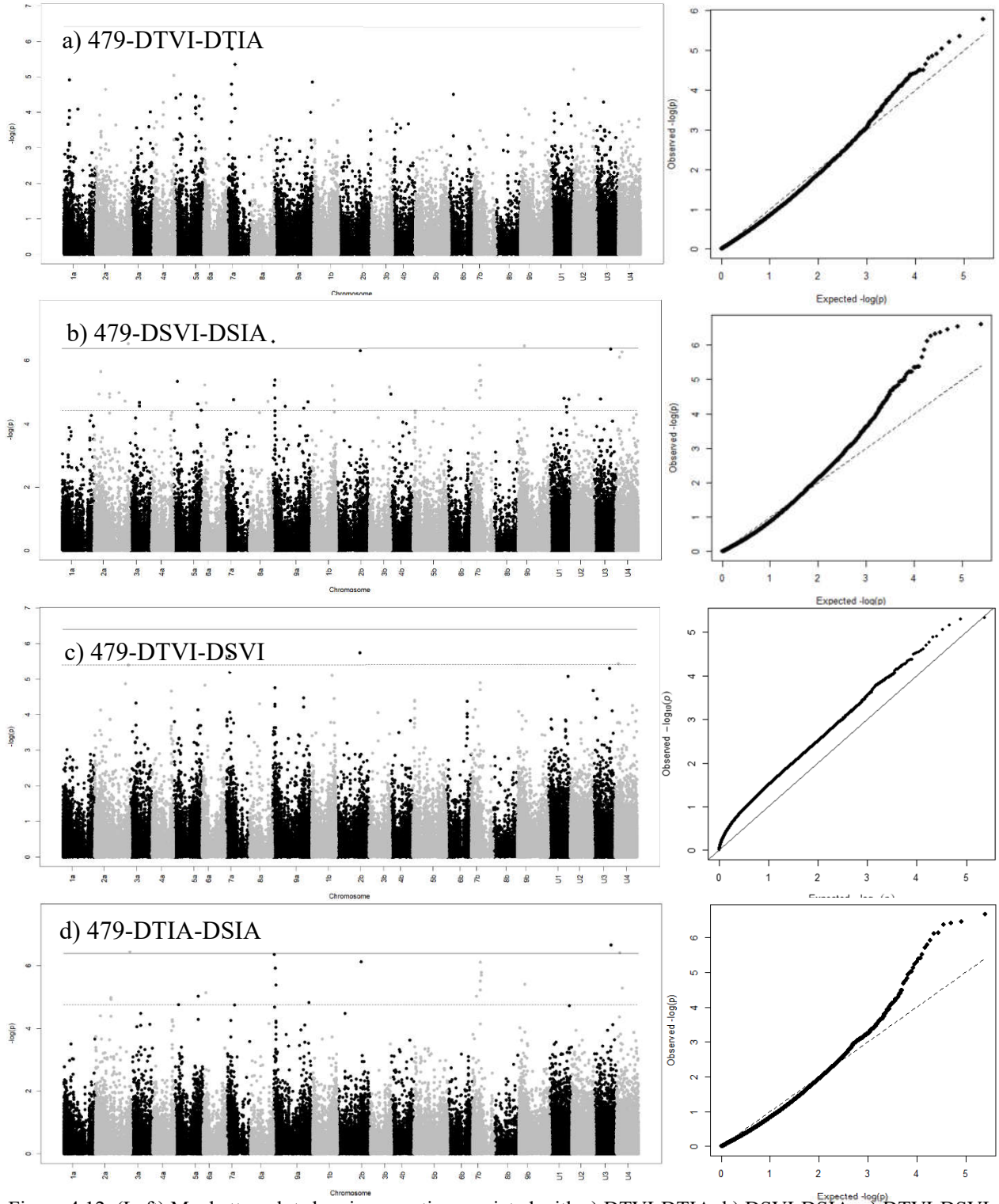


Figure 4.12. (Left) Manhattan plot showing genetic associated with a) DTVI-DTIA, b) DSVI-DSIA, c) DTVI-DSVI and d) DTIA-DSIA in 479 genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in 479 genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via vision (DSVI), and BLUPs of severity from the leaf disk assay via image analysis (DSIA).

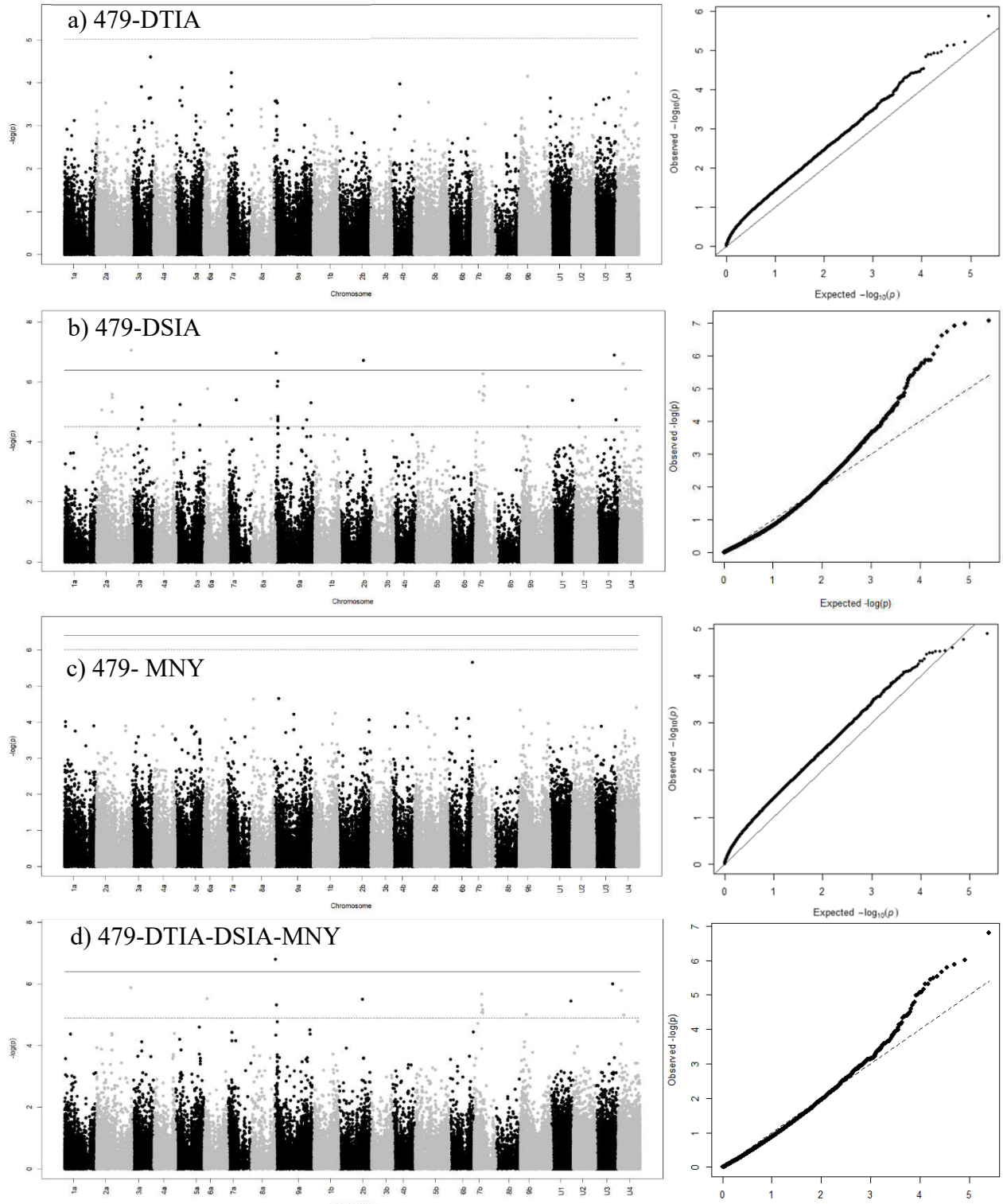


Figure 4.13. (Left) Manhattan plot showing genetic associated with a) DTIA, b) DSIA, c) MNY and d) DTIA-DSIA-MNY in 479 genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U2. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in 479 genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), and highest score of BLS in NY (MNY).

In a tetraploid group, the same three-trait combination of DTIA-DSIA-MNY did not provide the better GWAS than MNY alone (Figure 4.14c, d). Among all the trait combinations in the tetraploids, MNY provided the best association analysis with the expected distribution. However, none of GWAS octaploid group provided significant GWAS with reliable QQ-plots. In lowland group, the most reliable GWAS was from the combination of DTIA-DSIA-MNY-MPA (Figure 4.15d). Interestingly, in the upland group, none of the image analysis traits of DTIA and DSIA provided a reliable GWAS. The only nearly reliable single-trait GWAS in the upland group was DTVI with the convex line in the middle of the QQ-plot (Figure 4.16a). The two-trait combination of DTVI-MNY provided the most reliable GWAS with the expected QQ-plot (Figure 4.16c). In contrast to the full set of 479 genotypes, the inclusion of the trait from the leaf disk assay did not improve the analysis (Figure 4.16d).

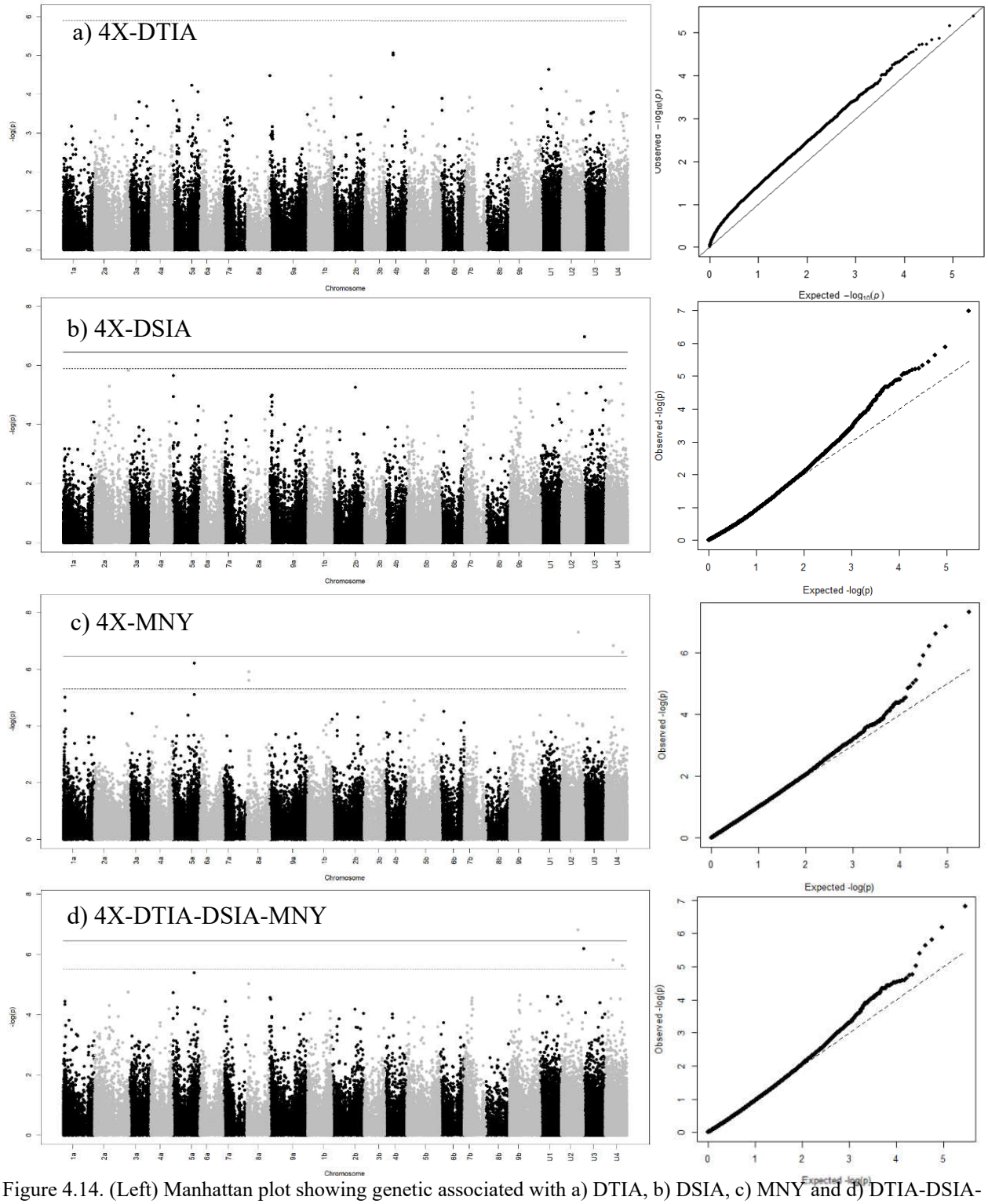


Figure 4.14. (Left) Manhattan plot showing genetic associated with a) DTIA, b) DSIA, c) MNY and d) DTIA-DSIA-MNY in 4X genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in 4X genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), and highest score of BLS in NY (MNY).

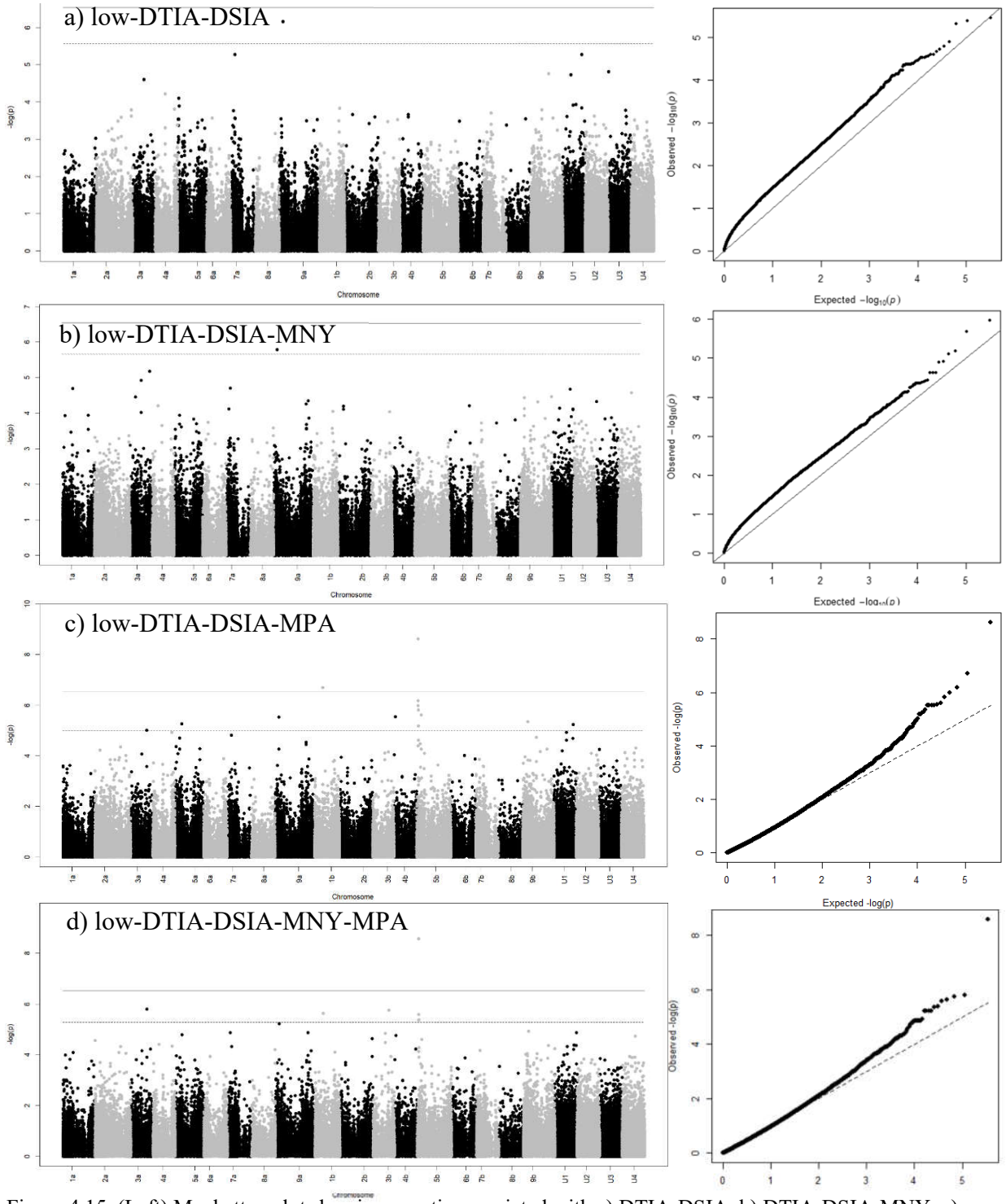


Figure 4.15. (Left) Manhattan plot showing genetic associated with a) DTIA-DSIA, b) DTIA-DSIA-MNY, c) DTIA-DSIA-MPA and d) DTIA-DSIA-MNY-MPA in lowland genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in lowland genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), and highest score of BLS in NY (MNY), highest score of BLS in PA (MPA).

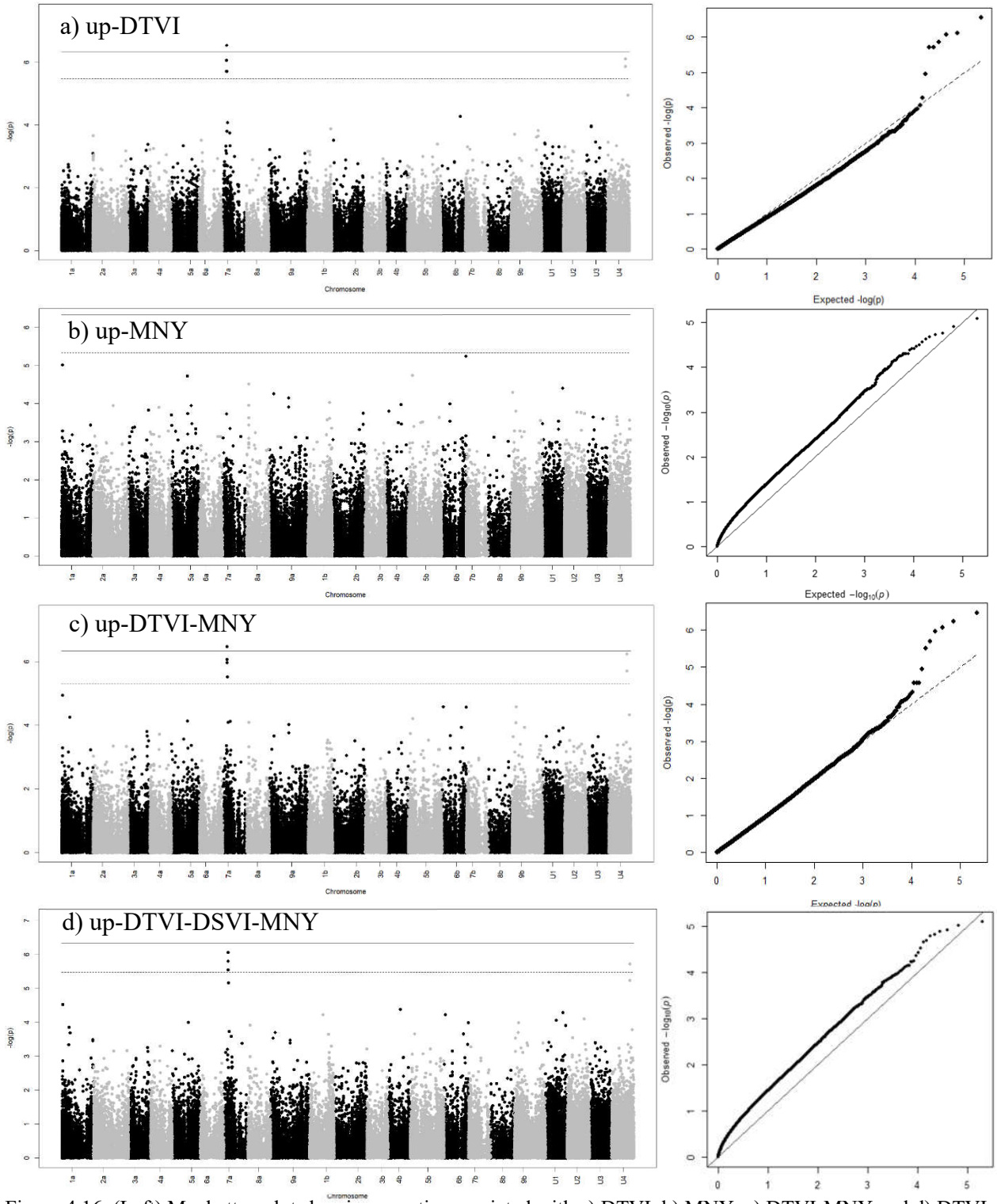


Figure 4.16. (Left) Manhattan plot showing genetic associated with a) DTVI, b) MNY, c) DTVI-MNY and d) DTVI-DSVI-MNY in upland genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (Right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in upland genotypes.



Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf disk assay via vision (DSVI), and highest score of BLS in NY (MNY).

In this study, therefore, we focused on four reliable GWAS analyses including 479-DTIA-DSIA-MNY, 4X-MNY, low-DTIA-DSIA-MNY-MPA, and up-DTVI-MNY (Figure 4.17). However, there was no overlapping region associated with the resistance among the four GWAS analyses (Table 4.3). Accumulatively, when considering phenotypic variance explained (PVE) by all 18 significant SNP markers for each trait, PVE of DSIA was the highest at 26.72% and PVE of DTIA was the lowest at 6.32% (Table 4.4). In 479-DTIA-DSIA-MNY, the significant markers were on chromosomes 2b, 6a, 7b, 9a, and 9b. The most significant marker on chromosome 2a was snp680175 followed by snp151084. Only snp359167 was the significant marker on chromosome 6a. The most significant marker on chromosome 7b was snp938364 followed by snp933838. The most significant marker on chromosome 9a was snp469523 followed by snp465249. The snp1003871 was the only significant marker on chromosome 9b. These eight SNP markers explained phenotypic variances for DTIA, DSIA and MNY of 2.1, 24.28, and 5.27%, respectively (Table 4.5). In 4X-MNY, there were only two significant markers snp316732 on chromosome 5a and snp444058 on chromosome 8a, accumulatively explaining 1.58% in MNY. In low-DTIA-DSIA-MNY-MPA, the significant markers were on chromosomes 1b, 3a, 3b and 5b. Only snp594527 was the significant marker on chromosome 1b. Also, snp197006 was the only significant marker on chromosome 3a, and snp732235 was the only one on chromosome 3b. The most significant marker on chromosome 5b was snp791318 followed by snp791375. These five SNP markers explained phenotypic variances for DTIA, DSIA, MNY, and MPA of 2.41, 8.31, 6.86, and 32.48%, respectively. In up-DTVI-MNY, the significant markers were on only chromosome 7a, in which snp411995 was the most significant marker, followed by snp411929 and snp412960. Among these markers, only snp938364, on chromosome 7b from

479-DTIA-DSIA-MNY, was linked with two genes: Pavir.Gb01488.1 and Pavir.Gb01488.2. These two were the only genes that overlapped with LOC_Os04g39430.1, one of the potential candidate genes for resistance to BLS in rice on chromosome 4 from AE11005627 marker (Table 4.6). Also, these three SNP markers explained phenotypic variances for DTVI and MNY of 7.19 and 0.27%, respectively. The PVEs of each SNP in each subgroup and each trait are reported in the appendix.

From the QTLs study of Sato et al. (2015), the candidate resistance genes in rice were reported to be on 3 chromosomes with 13 SNPs markers within the LD of 200 kb (Table 4.6). There were 278 genes linked to these markers. Among them, 172 genes were annotated with 134 biological functions. There were 106 non-annotated genes. The most frequent biological functions were Leucine-rich repeat family protein (6), peroxidase precursor (6), transposon protein (6), nucleotide binding sites – leucine rice repeats (NBS-LRR) type disease resistance protein (5), MYB family transcription factor (3), dirigent (2), fasciclin domain-containing protein (2), flavin monooxygenase (2), and heavy metal associated domain (2).

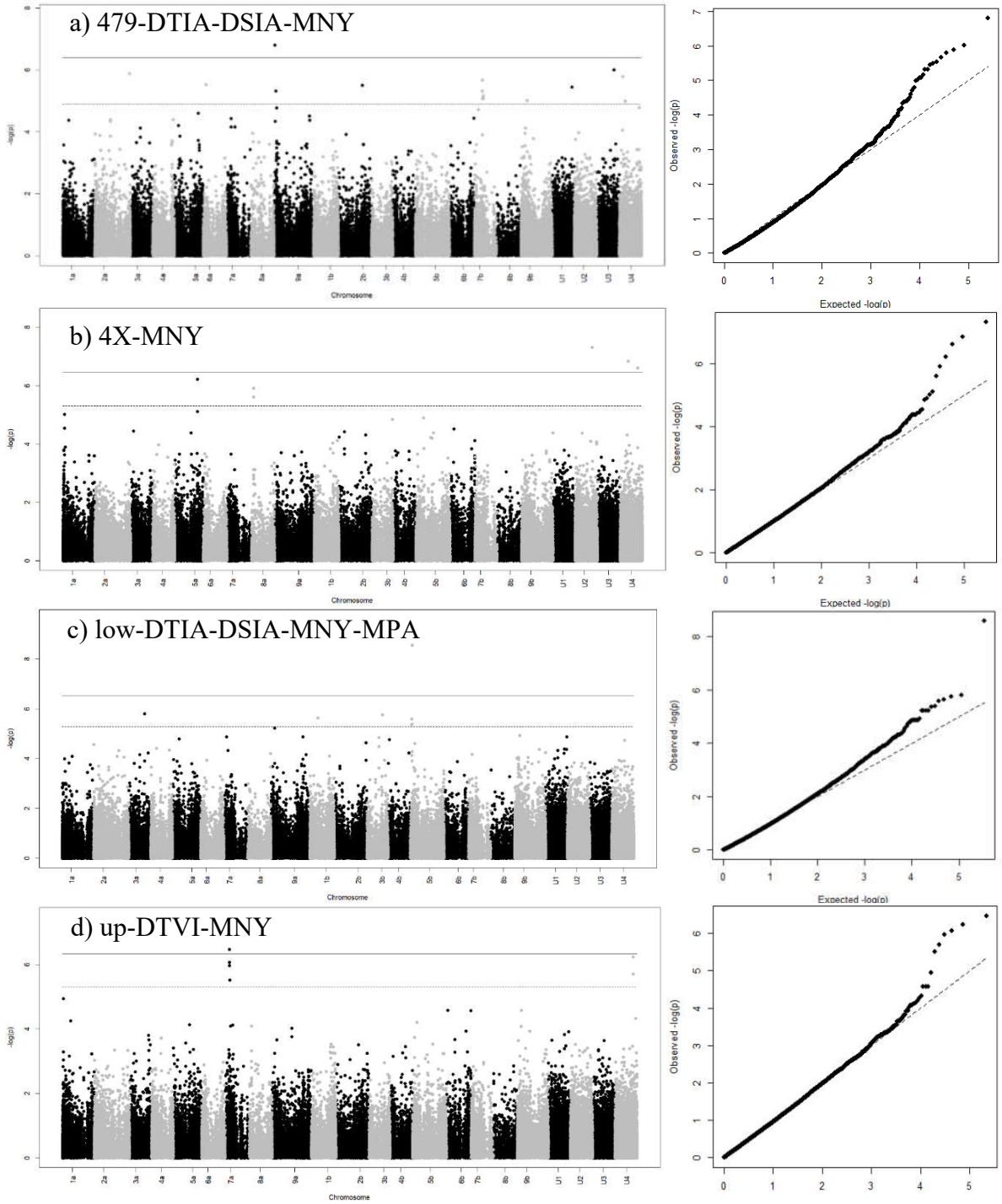


Figure 4.17. (Left) Manhattan plot showing genetic associated of a) DTIA-DSIA-MNY in 479 genotypes, b) MNY in 4X genotypes, c) DTIA-DSIA-MNY-MPA in lowland ecotype and d) DTVI-MNY in upland genotypes. The dashed line represents the FDR threshold (0.1) and the line represents Bonferroni correction threshold. On x-axis, the physical positions of the SNPs were aligned in 18 chromosomes of *P. virgatum*; however, there were 16,676 genes that cannot be assigned to the genome and noted as chromosome U1-U4. (right) Quantile-quantile (QQ) plots between the distributions of observed to expected P-values for GWAS of each trait combination in each genotype group.



Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), highest score of BLS in NY (MNY), highest score of BLS in PA (MPA).

Table 4.3. Markers significantly associated with resistance in each case.

| Cases | SNP | Chromosome | Distance from gene (bp) | p-value | *P. virgatum* gene | Orthologous genes | Identity | Predicted function |
|---|---|---|---|---|---|---|---|---|
| 479-DTIA-DSIA-MNY | snp151084 | Chr02a | 419 | 1.32E-06 | Pavir.Ba03861.1 | Pahal.B02018.1[Pha] | 99.4 | copper transport protein ATOX1-related |
| | snp680175 | Chr02b | 975 | 3.17E-06 | Pavir.Bb02195.1 | Pahal.B02834.1[Pha] | 94.8 | E3 ubiquitin-protein ligase RNF144 [EC:6.3.2.19] |
| | | | 3533 | | Pavir.Bb02196.1 | Pahal.J00890.1[Pha] | 93.8 | RBR family ring finger and IBR domain-containing |
| | snp359167 | Chr06a | 2850 | 2.96E-06 | Pavir.Fa00433.1<br>Pavir.Fa00433.2 | Pahal.F00718.1[Pha] | 88.9 | ENT domain |
| | | | 716 | | Pavir.Fa00434.1 | Pahal.F00719.1[Pha] | 70.9 | Myb-like DNA- binding domain, Bromodomain |
| | | | 2690 | | Pavir.Fa00435.1 | Pahal.F00721.1[Pha] | 54.6 | uncharacterized C-terminal membrane protein |
| | | | 8477 | | Pavir.Fa00437.1 | LOC_Os04g48030.1[Osa] | 8.5 | heat stress transcription factor B-1 |
| | snp933838 | Chr07b | 1453 | 2.12E-06 | Pavir.Gb01298.1<br>Pavir.Gb01298.2 | Sobic.006G139100.1[Sbi] | 96.7 | polyadenylate-binding protein |
| | | | 609 | | Pavir.Gb01299.1 | Pahal.H00668.1[Pha] | 93.5 | 2,3-dimethylmalate lyase, (2R,3S)-2,3-dimethylmalate pyruvate-lyase, isocitrate lyase |
| | snp938364 | Chr07b | 597 | 6.81E-06 | Pavir.Gb01488.1*<br>Pavir.Gb01488.2* | LOC_Os04g39430.1[Osa] | 85.6 | cytochrome P450 |
| | | | 2987 | | Pavir.Gb01489.1<br>Pavir.Gb01489.2<br>Pavir.Gb01489.3<br>Pavir.Gb01489.4 | Pahal.G00545.1[Pha] | 97.4 | protection of telomeres 1 |
| | snp465249 | Chr09a | 3464 | 1.58E-07 | Pavir.Ia00174.1 | Pahal.I01213.1[Pha] | 97.3 | protein trichome Birefringence-like 32 |

| Cases | SNP | Chromosome | Distance from gene (bp) | p-value | *P. virgatum* gene | Orthologous genes | Identity | Predicted function |
|---|---|---|---|---|---|---|---|---|
| | | | 1915 | | Pavir.Ia00175.1<br>Pavir.Ia00175.2 | Sobic.001G038100.1[Sbi] | 92.2 | protein FLC expressor |
| | snp469523 | Chr09a | 5412 | 4.77E-06 | Pavir.Ia00350.2 | Pahal.I01603.1[Pha] | 89.4 | protein K03B8.4-related |
| | | | 2256 | 4.77E-06 | Pavir.Ia00351.1 | Sobic.001G079200.1[Sbi] | 97.3 | mitogen-activated kinase kinase kinase (MAP) |
| | snp1003871 | Chr09b | 5314 | 9.85E-06 | Pavir.Ib01315.1<br>Pavir.Ib01315.2 | Sevir.9G450400.1[Svi] | 95.0 | glutathione s-transferase (GST) |
| | | | 788 | | Pavir.Ib01317.1 | Pahal.I04479.1[Pha] | 94.1 | glutathione s-transferase (GST) |
| | | | 3573 | | Pavir.Ib01318.1 | Zm00008a00114T01[Zma] | 83.0 | ent-kaurenoic acid hydroxylase (KAO) subfamily P450 |
| 4X-MNYNY | snp316732 | Chr05a | 8347 | 6.08E-07 | Pavir.Ea02827.1 | Sevir.3G000300.1[Svi] | 63.6 | Actinidain/Actinidin [3.4.22.14], cysteine protease, Cathepsin propeptide inhibitor domain |
| | | | 2009 | | Pavir.Ea02828.1<br>Pavir.Ea02828.2<br>Pavir.Ea02828.3 | Pahal.E01498.1[Pha] | 96.3 | [3.64.13] RNA helicase, ATP-dependent RNA helicase, DEAD/DEAH box helicase |
| | snp444058 | Chr08a | 1739 | 1.21E-06 | Pavir.Ha00272.1 | Pahal.H00237.1[Pha] | 90.6 | auxilin/cyclin g-associated kinase-related |
| low-DTIA-DSIA-MNY-MPA | snp594527 | Chr01b | 7186 | 2.37E-06 | Pavir.Ab01193.1<br>Pavir.Ab01193.2<br>Pavir.Ab01193.3 | LOC_Os07g35340.1[Osa] | 74.7 | protein kinase domain (Pkinase), salt stress response,antifungal (stress-antifung) |
| | | | 199 | | Pavir.Ab01194.1 | Pahal.A00997.1[Pha] | 56.0 | component of root nitrate treatment specific coexpression subnetwork |
| | snp197006 | Chr03a | 1106 | 1.58E-06 | Pavir.Ca02197.1 | Sobic.008G093000.1[Sbi] | 92.6 | ATP-binding cassette (ABC) transporter |

| Cases | SNP | Chromosome | Distance from gene (bp) | p-value | *P. virgatum* gene | Orthologous genes | Identity | Predicted function |
|---|---|---|---|---|---|---|---|---|
| | snp732235 | Chr03b | 1613 | 1.75E-06 | Pavir.Cb01411.1<br>Pavir.Cb01411.2 | Zm00008a03100T01[Zma] | 99.8 | elongation factor 1-alpha (EEF1A) |
| | | | 4102 | | Pavir.Cb01412.1 | Sobic.009G118300.1[Sbi] | 95.1 | copine |
| | snp791318 | Chr05b | 7974 | 2.56E-09 | Pavir.Eb00172.1 | Sevir.5G014600.1[Svi] | 82.6 | protein kinase domain (Pkinase), S-locus glycoprotein domain, D-mannose binding lectin (B_lectin), PAN-like domain (PAN_2), serine/threonine protein kinase |
| | | | 5076 | | Pavir.Eb00173.1<br>Pavir.Eb00173.2 | Pahal.E04515.1[Pha] | 97.7 | HAT family dimerization domain-containing protein, BED zinc finger |
| | | | 1362 | | Pavir.Eb00174.1 | Pahal.E04517.1[Pha] | 98.3 | Diacylglycerol o-acyltransferase 1 (DGAT1) |
| | | | 2273 | | Pavir.Eb00175.1 | Sobic.003G013800.1[Sbi] | 88.0 | uncharacterized |
| | snp791375 | Chr05b | 3373 | 4.16E-06 | Pavir.Eb00177.1 | Sobic.003G014100.1[Sbi] | 64.8 | GDT1-like protein 1, chloroplastic, membrane protein |
| | | | 4141 | | Pavir.Eb00178.1 | Sobic.003G014300.2[Sbi] | 99.5 | Snoal-like domain, uncharacterized conserved protein |
| up-DTVI-MNY | snp411929 | Chr07a | 2184 | 3.44E-07 | Pavir.Ga00855.1<br>Pavir.Ga00855.2<br>Pavir.Ga00855.3 | Pahal.G01363.1[Pha] | 96.8 | RNA polymerase II, C-terminal domain phosphatase-like 1/2 [EC:3.1.3.16] (CPL1_2), protein-serine/threonine phosphatase |
| | | | 6254 | | Pavir.Ga00856.1 | Zm00008a03828T01[Zma] | 74.2 | lactosylceramide 4-alpha-galactosyltransferase |
| | snp411995 | Chr07a | 2093 | 8.66E-07 | Pavir.Ga00857.1 | Sobic.006G156600.2[Sbi] | 97.2 | transporter family protein |

| Cases | SNP | Chromosome | Distance from gene (bp) | p-value | *P. virgatum* gene | Orthologous genes | Identity | Predicted function |
|---|---|---|---|---|---|---|---|---|
| | | | 507 | | Pavir.Ga00858.1 | Sobic.006G156600.2[Sbi] | 54.0 | sugar (and other) transporter |
| | | | 49 | | Pavir.Ga00859.1<br>Pavir.Ga00859.2<br>Pavir.Ga00859.3<br>Pavir.Ga00859.4<br>Pavir.Ga00859.5<br>Pavir.Ga00859.6 | Zm00008a00013T01[Zma] | 76.8 | general transcription factor 3c polypeptide 6 (GTF3C6) |
| | | | 6080 | | Pavir.Ga00860.1<br>Pavir.Ga00860.2 | Zm00008a00671T01[Zma] | 92.2 | expansin precursor |
| | snp412960 | Chr07a | 673 | 3.08E-06 | Pavir.Ga00912.1<br>Pavir.Ga00912.2 | Sobic.006G156300.1[Sbi] | 92.7 | WD-40 repeat family protein |

[Osa] gene from *Oryza sativa*

[Pha] gene from Panicum hallii

[Sbi] gene from Sorghum bicolor

[Svi] gene from Setaria viridis

[Zma] gene from Zea may

Table 4.4 Phenotypic variance explained (PVE) (adjusted PVE) by accumulative 18 significant SNP markers across all analyses for each trait.

| Traits | PVE (adjusted PVE) |
|---|---|
| DTVI | 7.68 (4.07) |
| DTIA | 6.32 (2.65) |
| DSIA | 26.72 (23.85) |
| MNY | 9.93 (6.4) |
| MPA | 11.38 (7.91) |

Table 4.5 Phenotypic variance explained (PVE) (adjusted PVE) by set of significant SNP markers in each subgroup for traits used in each subgroup.

| Subgroups | Traits | PVE (adjusted PVE) |
|---|---|---|
| 479 | DTIA | 2.1 (0.44) |
| | DSIA | 24.28 (22.99) |
| | MNY | 5.27 (3.66) |
| 4X | MNY | 1.58 (0.85) |
| low | DTIA | 2.41 (-1.35) |
| | DSIA | 8.31 (4.79) |
| | MNY | 6.85 (3.26) |
| | MPA | 32.48 (29.88) |
| up | DTVI | 7.19 (6.39) |
| | MNY | 0.27 (-0.59) |

Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), highest score of BLS in NY (MNY), highest score of BLS in PA (MPA).

Table 4.6. Candidate genes and their annotated functions for resistance to BLS in rice (*Oryza sativa*) from QTLs from Sato et al. (2015) within LD of 200 kb.

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| Chr1 | AE01003285 (7.8) | peroxidase precursor | 6 | LOC_Os01g18890.1, LOC_Os01g18910.1, LOC_Os01g18930.1, LOC_Os01g18950.1, LOC_Os01g18970.1, LOC_Os01g19020.1 |
| | | expressed protein | 3 | LOC_Os01g18810.1, LOC_Os01g18830.1, LOC_Os01g18940.1 |
| | | transposon protein CACTA En/Spm sub-class | 2 | LOC_Os01g19070.1, LOC_Os01g19110.1 |
| | | CAMK_KIN1/SNF1/Nim1_like.9 - CAMK includes calcium/calmodulin depedent protein kinases | 1 | LOC_Os01g18800.1 |
| | | hAT dimerisation domain-containing protein | 1 | LOC_Os01g18920.1 |
| | | helix-loop-helix DNA-binding domain containing protein | 1 | LOC_Os01g18870.1 |
| | | hypothetical protein | 1 | LOC_Os01g18960.1 |
| | | IF | 1 | LOC_Os01g18840.1 |
| | | ligA | 1 | LOC_Os01g19080.1 |
| | | OsSPL1 - SBP-box gene family member | 1 | LOC_Os01g18850.1 |
| | | S-adenosylmethionine synthetase | 1 | LOC_Os01g18860.1 |
| | | stigma specific peroxidase precursor | 1 | LOC_Os01g18900.1 |
| | | transposon protein unclassified | 1 | LOC_Os01g19050.1 |
| | | ubiquitin carboxyl-terminal hydrolase family 1 | 1 | LOC_Os01g18880.2 |
| | P0634_1 (9.5) | expressed protein | 13 | LOC_Os01g13780.1, LOC_Os01g13810.1, LOC_Os01g13830.1, LOC_Os01g13850.1, LOC_Os01g13860.1, LOC_Os01g13870.1, LOC_Os01g13880.1, LOC_Os01g13910.1, LOC_Os01g13930.1, LOC_Os01g13940.1, LOC_Os01g14000.1, LOC_Os01g14020.1, LOC_Os01g14060.1 |
| | | 60S ribosomal protein L18a-1 | 1 | LOC_Os01g14070.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | BT1 family protein | 1 | LOC_Os01g14100.1 |
| | | C2 domain containing protein | 1 | LOC_Os01g14050.1 |
| | | cysteine protease | 1 | LOC_Os01g13920.1 |
| | | D-tyrosyl-tRNA | 1 | LOC_Os01g14040.1 |
| | | dnaJ domain containing protein | 1 | LOC_Os01g13760.1 |
| | | DUF250 domain containing protein | 1 | LOC_Os01g13770.1 |
| | | DUF260 domain containing protein | 1 | LOC_Os01g14030.1 |
| | | kinesin motor domain containing protein | 1 | LOC_Os01g14090.1 |
| | | lipase class 3 family protein | 1 | LOC_Os01g14080.1 |
| | | OsGrx_A1 - glutaredoxin subgroup III | 1 | LOC_Os01g13950.1 |
| | | PE-PGRS family protein | 1 | LOC_Os01g13840.1 |
| | | receptor-like protein kinase 5 precursor | 1 | LOC_Os01g13800.1 |
| | | retrotransposon protein unclassified | 1 | LOC_Os01g13960.1 |
| | | ZOS1-05 - C2H2 zinc finger protein | 1 | LOC_Os01g14010.1 |
| | ad01004243 10.0) | expressed protein | 5 | LOC_Os01g16590.1, LOC_Os01g16600.1, LOC_Os01g16620.4, LOC_Os01g16630.1, LOC_Os01g16800.1 |
| | | flavin monooxygenase | 2 | LOC_Os01g16714.1, LOC_Os01g16750.1 |
| | | antifreeze glycoprotein | 1 | LOC_Os01g16560.1 |
| | | BSD domain-containing protein | 1 | LOC_Os01g16670.1 |
| | | expansin precursor | 1 | LOC_Os01g16770.1 |
| | | MYB family transcription factor | 1 | LOC_Os01g16810.1 |
| | | plastocyanin-like domain containing protein | 1 | LOC_Os01g16610.1 |
| | | split hand/foot malformation type 1 | 1 | LOC_Os01g16640.1 |
| | | transposon protein unclassified | 1 | LOC_Os01g16660.1 |
| | | ubiquitin-conjugating enzyme | 1 | LOC_Os01g16650.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | ulp1 protease family C-terminal catalytic domain containing protein | 1 | LOC_Os01g16730.1 |
| | ah01000747 (10.7) | expressed protein | 6 | LOC_Os01g17300.1, LOC_Os01g17396.2, LOC_Os01g17410.1, LOC_Os01g17420.1, LOC_Os01g17980.1, LOC_Os01g17990.1 |
| | | BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor | 1 | LOC_Os01g17250.1 |
| | | coatomer subunit beta-1 | 1 | LOC_Os01g17430.1 |
| | | cyclin | 1 | LOC_Os01g17402.1 |
| | | eukaryotic translation initiation factor 6 | 1 | LOC_Os01g17330.2 |
| | | exonuclease | 1 | LOC_Os01g17279.1 |
| | | hypothetical protein | 1 | LOC_Os01g18010.1 |
| | | major facilitator superfamily antiporter | 1 | LOC_Os01g17214.1 |
| | | OsFBX5 - F-box domain containing protein | 1 | LOC_Os01g17390.1 |
| | | plastocyanin-like domain containing protein | 1 | LOC_Os01g17470.1 |
| | | PPR repeat domain containing protein | 1 | LOC_Os01g17320.1 |
| | | retrotransposon protein unclassified | 1 | LOC_Os01g17310.1 |
| | | transcription factor | 1 | LOC_Os01g17260.2 |
| | | transporter major facilitator family | 1 | LOC_Os01g17240.1 |
| | | tubulin/FtsZ domain containing protein | 1 | LOC_Os01g18050.1 |
| Chr4 | P0709_2 (19.7) | expressed protein | 4 | LOC_Os04g32580.1, LOC_Os04g32600.1, LOC_Os04g32610.2, LOC_Os04g32690.1 |
| | | hypothetical protein | 2 | LOC_Os04g32640.1, LOC_Os04g32780.1 |
| | | 40S ribosomal protein S27 | 1 | LOC_Os04g32710.1 |
| | | AP2 domain containing protein | 1 | LOC_Os04g32790.1 |
| | | ATP-dependent Clp protease ATP-binding subunit clpA homolog CD4B chloroplast precursor | 1 | LOC_Os04g32560.1 |
| | | basic proline-rich protein | 1 | LOC_Os04g32800.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | cytochrome b-c1 complex subunit Rieske mitochondrial precursor | 1 | LOC_Os04g32660.1 |
| | | defender against cell death 1 | 1 | LOC_Os04g32550.1 |
| | | ethylene-responsive transcription factor ERF114 | 1 | LOC_Os04g32620.1 |
| | | exostosin family domain containing protein | 1 | LOC_Os04g32670.1 |
| | | hydrolase NUDIX family domain containing protein | 1 | LOC_Os04g32740.1 |
| | | LEML3 - Anther-specific LEM1 family protein precursor | 1 | LOC_Os04g32700.1 |
| | | MYB protein | 1 | LOC_Os04g32570.1 |
| | | POEI20 - Pollen Ole e I allergen and extensin family protein precursor | 1 | LOC_Os04g32680.1 |
| | | transcription factor | 1 | LOC_Os04g32590.1 |
| | | tRNA synthetase class II core domain containing protein | 1 | LOC_Os04g32650.1 |
| | ad04008446 (22.2) | expressed protein | 8 | LOC_Os04g36670.1, LOC_Os04g36780.1, LOC_Os04g36820.1, LOC_Os04g36880.1, LOC_Os04g36900.1, LOC_Os04g36910.1, LOC_Os04g37410.1, LOC_Os04g37420.1 |
| | | 3-oxoacyl-synthase | 1 | LOC_Os04g36800.1 |
| | | calmodulin binding protein | 1 | LOC_Os04g36660.1 |
| | | DUF567 domain containing protein | 1 | LOC_Os04g36830.1 |
| | | enzyme of the cupin superfamily protein | 1 | LOC_Os04g36760.1 |
| | | ferric-chelate reductase | 1 | LOC_Os04g36720.1 |
| | | hsp20/alpha crystallin family protein | 1 | LOC_Os04g36750.1 |
| | | LSM domain containing protein | 1 | LOC_Os04g36810.1 |
| | | methyltransferase domain containing protein | 1 | LOC_Os04g36710.1 |
| | | MLO domain containing protein | 1 | LOC_Os04g36680.1 |
| | | ORG4 | 1 | LOC_Os04g36850.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | peptidyl-prolyl cis-trans isomerase FKBP-type | 1 | LOC_Os04g36890.1 |
| | | PHD finger protein | 1 | LOC_Os04g36730.1 |
| | | PME/invertase inhibitor | 1 | LOC_Os04g36770.1 |
| | | potassium channel SKOR | 1 | LOC_Os04g36740.1 |
| | | PPR repeat domain containing protein | 1 | LOC_Os04g36840.1 |
| | | proteasome subunit | 1 | LOC_Os04g36700.1 |
| | | signal peptidase complex subunit 2 | 1 | LOC_Os04g36859.1 |
| | | transcription factor like protein | 1 | LOC_Os04g36790.1 |
| | | ZOS4-06 - C2H2 zinc finger protein | 1 | LOC_Os04g36650.1 |
| | ad04008616 (23.5) | expressed protein | 8 | LOC_Os04g39320.1, LOC_Os04g39330.1, LOC_Os04g39340.1, LOC_Os04g39450.1, LOC_Os04g39510.1, LOC_Os04g39540.1, LOC_Os04g39560.1, LOC_Os04g39580.1 |
| | | fasciclin domain containing protein | 2 | LOC_Os04g39590.1, LOC_Os04g39600.1 |
| | | heavy metal associated domain containing protein | 2 | LOC_Os04g39350.1, LOC_Os04g39370.1 |
| | | heavy metal transport/detoxification protein | 2 | LOC_Os04g39360.1, LOC_Os04g39380.1 |
| | | 6-phosphofructokinase 2 | 1 | LOC_Os04g39420.1 |
| | | amino acid transporter | 1 | LOC_Os04g39489.1 |
| | | cytochrome P450 | 1 | LOC_Os04g39430.1 |
| | | LSM domain containing protein | 1 | LOC_Os04g39444.1 |
| | | MYB family transcription factor | 1 | LOC_Os04g39470.1 |
| | | NBS-LRR type disease resistance protein | 1 | LOC_Os04g39460.1 |
| | | pentatricopeptide | 1 | LOC_Os04g39410.1 |
| | | ras-related protein | 1 | LOC_Os04g39440.1 |
| | | transposon protein CACTA En/Spm sub-class | 1 | LOC_Os04g39530.1 |
| | | WRKY35 | 1 | LOC_Os04g39570.1 |
| | | ZOS4-08 - C2H2 zinc finger protein | 1 | LOC_Os04g39520.1 |

| Chrom osomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | ad04009558 (25.8) | expressed protein | 5 | LOC_Os04g43450.1, LOC_Os04g43519.1, LOC_Os04g43570.1, LOC_Os04g43660.1, LOC_Os04g43670.1 |
| | | CAMK_CAMK_like.3 - CAMK includes calcium/calmodulin depedent protein kinases | 1 | LOC_Os04g43710.1 |
| | | CK1_CaseinKinase_1.7 - CK1 includes the casein kinase 1 kinases | 1 | LOC_Os04g43490.1 |
| | | DUF640 domain containing protein | 1 | LOC_Os04g43580.2 |
| | | DUF647 domain containing protein | 1 | LOC_Os04g43690.1 |
| | | glycosyl transferase 8 domain containing protein | 1 | LOC_Os04g43700.1 |
| | | hypothetical protein | 1 | LOC_Os04g43640.1 |
| | | kinase pfkB family | 1 | LOC_Os04g43750.1 |
| | | L-allo-threonine aldolase | 1 | LOC_Os04g43650.2 |
| | | MazG nucleotide pyrophosphohydrolase domain containing protein | 1 | LOC_Os04g43550.1 |
| | | MYB family transcription factor | 1 | LOC_Os04g43680.1 |
| | | NB-ARC/LRR disease resistance protein | 1 | LOC_Os04g43440.1 |
| | | no apical meristem protein | 1 | LOC_Os04g43560.1 |
| | | OsSAUR18 - Auxin-responsive SAUR gene family member | 1 | LOC_Os04g43740.1 |
| | | OsWAK51 - OsWAK receptor-like protein kinase | 1 | LOC_Os04g43730.1 |
| | | pentatricopeptide | 1 | LOC_Os04g43430.2 |
| | | PTAC5 | 1 | LOC_Os04g43420.1 |
| | | retrotransposon protein unclassified | 1 | LOC_Os04g43480.1 |
| | | transposon protein CACTA En/Spm sub-class | 1 | LOC_Os04g43590.1 |
| Chr11 | AE11002117 (17.9) | expressed protein | 11 | LOC_Os11g30620.1, LOC_Os11g30640.1, LOC_Os11g30690.1, LOC_Os11g30750.1, LOC_Os11g30760.1, LOC_Os11g30770.1, LOC_Os11g30780.1, LOC_Os11g30790.1, |

| Chrom osomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | | | LOC_Os11g30820.1, LOC_Os11g30830.1, LOC_Os11g30930.1 |
| | | conserved hypothetical protein | 2 | LOC_Os11g30660.1, LOC_Os11g30720.1 |
| | | sulfotransferase domain containing protein | 2 | LOC_Os11g30810.1, LOC_Os11g30910.1 |
| | | hypothetical protein | 1 | LOC_Os11g30600.1 |
| | | transposon protein CACTA En/Spm sub-class | 1 | LOC_Os11g30740.1 |
| | ah11000824 (21.0) | expressed protein | 8 | LOC_Os11g35670.1, LOC_Os11g35720.1, LOC_Os11g35750.1, LOC_Os11g35810.1, LOC_Os11g35820.1, LOC_Os11g35830.1, LOC_Os11g35840.1, LOC_Os11g35850.1 |
| | | cycloartenol synthase | 1 | LOC_Os11g35710.1 |
| | | hypothetical protein | 1 | LOC_Os11g35900.1 |
| | | leucine-rich repeat receptor protein kinase EXS precursor | 1 | LOC_Os11g35660.1 |
| | | Leucine rich repeat N-terminal domain containing protein | 1 | LOC_Os11g35790.1 |
| | | leucine rich repeat protein | 1 | LOC_Os11g35890.1 |
| | | OsWAK120 - OsWAK receptor-like protein kinase | 1 | LOC_Os11g35860.1 |
| | | retrotransposon protein unclassified | 1 | LOC_Os11g35690.1 |
| | | RWD domain containing protein | 1 | LOC_Os11g35870.1 |
| | | transposon protein CACTA En/Spm sub-class | 1 | LOC_Os11g35762.1 |
| | P0511 (22.9) | expressed protein | 9 | LOC_Os11g38510.1, LOC_Os11g38520.1, LOC_Os11g38590.1, LOC_Os11g38610.2, LOC_Os11g38620.1, LOC_Os11g38630.1, LOC_Os11g38640.1, LOC_Os11g38680.1, LOC_Os11g38710.1 |
| | | NBS-LRR type disease resistance protein | 2 | LOC_Os11g38480.1, LOC_Os11g38580.1 |
| | | transposon protein CACTA En/Spm sub-class | 2 | LOC_Os11g38550.1, LOC_Os11g38690.1 |
| | | DEAD-box ATP-dependent RNA helicase | 1 | LOC_Os11g38670.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | OsFBDUF62 - F-box and DUF domain containing protein | 1 | LOC_Os11g38500.1 |
| | | TruB family pseudouridylate synthase | 1 | LOC_Os11g38600.1 |
| | | UDP-glucoronosyl/UDP-glucosyl transferase | 1 | LOC_Os11g38650.1 |
| | AE11005627 (25.3) | expressed protein | 9 | LOC_Os11g41920.1, LOC_Os11g41940.1, LOC_Os11g41950.1, LOC_Os11g42020.1, LOC_Os11g42030.1, LOC_Os11g42050.1, LOC_Os11g42080.1, LOC_Os11g42110.1, LOC_Os11g42120.1 |
| | | Leucine Rich Repeat family protein | 4 | LOC_Os11g42060.1, LOC_Os11g42070.1, LOC_Os11g42090.1, LOC_Os11g42100.1 |
| | | hypothetical protein | 3 | LOC_Os11g41960.1, LOC_Os11g41970.1, LOC_Os11g42150.1 |
| | | acyltransferase | 1 | LOC_Os11g41900.1 |
| | | armadillo/beta-catenin-like repeat family protein | 1 | LOC_Os11g41990.1 |
| | | F-box/LRR-repeat protein 3 | 1 | LOC_Os11g42160.1 |
| | | FRA10AC1 | 1 | LOC_Os11g42000.1 |
| | | GTP-binding protein | 1 | LOC_Os11g41910.1 |
| | | NBS-LRR type disease resistance protein | 1 | LOC_Os11g42010.1 |
| | | non-TIR-NBS-LRR type resistance protein | 1 | LOC_Os11g42040.1 |
| | ah11000941 (25.6) | expressed protein | 6 | LOC_Os11g42380.1, LOC_Os11g42400.1, LOC_Os11g42410.1, LOC_Os11g42440.1, LOC_Os11g42470.1, LOC_Os11g42590.1 |
| | | dirigent | 2 | LOC_Os11g42500.1, LOC_Os11g42550.1 |
| | | Leucine Rich Repeat family protein | 2 | LOC_Os11g42580.1, LOC_Os11g42660.1 |
| | | DEFL25 - Defensin and Defensin-like DEFL family | 1 | LOC_Os11g42530.1 |
| | | DEFL34 - Defensin and Defensin-like DEFL family | 1 | LOC_Os11g42520.1 |
| | | hypothetical protein | 1 | LOC_Os11g42525.1 |

| Chromosomes | Marker names (position Mb) | Annotated functions | #genes expressing same traits | Gene list |
|---|---|---|---|---|
| | | leucine-rich repeat family protein | 1 | LOC_Os11g42450.1 |
| | | NBS-LRR type disease resistance protein | 1 | LOC_Os11g42570.1 |
| | | nuclear pore protein 84/107 containing protein | 1 | LOC_Os11g42420.1 |
| | | OsSCP64 - Putative Serine Carboxypeptidase homologue | 1 | LOC_Os11g42390.1 |
| | | oxidoreductase aldo/keto reductase family protein | 1 | LOC_Os11g42540.1 |
| | | transferase family domain containing protein | 1 | LOC_Os11g42480.1 |
| | | transferase family protein | 1 | LOC_Os11g42370.1 |
| | | transporter family protein | 1 | LOC_Os11g42430.1 |
| | | transposon protein CACTA En/Spm sub-class | 1 | LOC_Os11g42610.1 |
| | | tyrosine aminotransferase | 1 | LOC_Os11g42510.1 |

## Discussion

### The most resistant population for improvement of resistance to BLS

Since the various phenotyping approaches yielded various resistant populations, it was challenging to determine the most resistant candidates for further breeding. Based on field evaluation, BLUPs from two locations were zero due to zero broad-sense heritability. This indicated huge GxE effects between two locations. If the mean of field scores were used, the upland Shelter should be considered the most resistant population across two locations with a mean of 0.44 from 5. The high resistance of Shelter from field evaluation may explain the reason in Chapter 3 that it cannot be improved for resistance to BLS in recurrent phenotypic selection in seedlings. However, the most susceptible SW787 had the mean score only 2.33. This suggested the low natural inoculation in 2017 which was not conducive to severe disease development. When comparing each location, the most resistant population in NY was SW803 at 0.22, but it was almost the most susceptible population in PA at 2.11. Therefore, this variation needs to be confirmed with more replicates, years, and locations.

To lessen the GxE effect on the BLS, the artificial inoculation was conducted in the laboratory. In leaf detachment assay, due to the limitation of sampling, a whole set of 479 genotype cannot be tested at the same time. The experiment was separated into seven overlapping sets. However, there was significant variation among sets. Such a variation can occur from the different age of each genotype in the same sampling time. Although the same stage of leaves at 30 centimeters from the top was sampled, there was variation among leaves from the same plant. To handle the

variation among sets, BLUPs were used by fitting the effect from the set as a fixed effect. Another artificial inoculation was leaf disk assay, without limitation of the size, the whole set of 479 genotypes was sampled at the same time with resistant and susceptible checks in each plate. Although there was a small effect of plates, BLUPs was fitted by considering plates as a fixed effect. Both artificial inoculations were evaluated via vision and image analysis. Although vision and image analysis results were highly correlated, they yielded a different result from association analysis. Mostly, image analysis provided more significant analysis. This could be explained by the more refined data from image analysis with decimals than vision analysis (which grouped into ten increments). Moreover, the image analysis is better regarding repeatability and reanalysis (Stewart and McDonald, 2014). For the sake of comparison for the most resistant population, mean of percent lesion from image analysis was considered. In the leaf detachment approach, the most resistant population from DTIA was ECS.6 at 8%, but the population appeared very susceptible from DSIA at 94%. In leaf disk assay, the most resistant population from DSIA was SW803 and SW793. These confirmed the low correlation among all three disease evaluation approaches. Regardless of low correlations, SW805 was the most resistant population across the mean of two locations, DTIA, and DSIA, 10% and 56% resistance, respectively, with mean of 0.78.

**The improvement of multi-trait GWAS from single trait GWAS**

Although all single traits from a different phenotype approach had moderate to high heritability, none of them provided reliable GWAS based on QQ-plots that were not distributed as expected from theory. This unreliability can be explained by the

high density of markers with high collinearity between markers, and too small population size giving the low power detection (Muir, 2007). The combination of the traits for resistance to BLS from different approaches helped improve the association analysis power (Guo et al., 2017). However, the addition of traits did not always improve the analysis. In the tetraploid groups, for example, only MNY yielded the most reliable GWAS. These suggested significant markers linked with the specific location. The spatial markers of simple sequence repeats (SSRs) in QTLs were also reported in resistance to BLS in rice (Katara et al., 2010). None of the reliable GWAS provides the same significant regions linked with the resistance to BLS. As those reliable GWASs were from a different subset of the association panel, it suggested different resistance allele distribution in the subgroups. It also suggests the multifaceted defense mechanism to BLS.

Despite the improvement of multi-trait GWAS over single-trait GWAS, 18 SNP markers can explain the phenotypic variance ranging from only 6.32 to 26.72% among DTVI, DTIA, DSIA, MNY and MPA. The low PVE from GWAS is a common phenomenon and generally caused by missing heritability and can be improved by more genomic imputation and implementation of a large-scale next-generation sequence data (Fan and Song, 2016).

Moreover, each genotype group can show reliable GWAS with specific traits. For example, the upland group only provided the reliable GWAS with DTVI-MNY, whereas the full set of 479 genotypes provided the best fit GWAS with DTIA-DSIA-MNY. To study further for resistance to BLS in switchgrass populations, single-trait GWAS is not suggested due to the low power of determining significant markers.

Multi-trait GWAS from both artificial and natural inoculation were suggested to improve the power. However, such an improvement cannot be applied to the octaploid population due to the uncertainty in differentiating heterozygote genotypes. The model for octaploid should be applied to the population instead of the diploid model to detect associations.

**Dissecting resistance to BLS**

As mentioned previously, none of the significant markers overlapped across reliable GWASs from different groups. Although this suggested the different distribution of resistance genes in different subgroups from the association panel, we considered the biological functions for the resistance over the fixation of the alleles in each population. In the full set, on chromosome 2b, Pavir.Ba03861.1 was predicted to function as a copper transport protein and as an antioxidant protein 1 (ATOX1). There was no study to confirm the function of this gene related to any plant disease resistance, but its antioxidant capacity may help handle ROS from the interaction between fungal effectors and plant response. Pavir.Bb02195.1 and Pavir.Bb02196.1 encoded RING-type E3 ubiquitin-protein ligase and RBR family ring finger, respectively, which collaboratively regulates cell death and immunity in rice (Smit and Sixma, 2014; You et al., 2016). On chromosome 6b, Pavir.Fa00433.1 encoded for the EMSY N-terminal (ENT) domain, which is important to mediate the race-specific NLR gene resistance to *Peronospora parasitica* (*RPP7*) gene in *Arabidopsis thaliana* (Tsuchiya and Eulgem, 2011). Moreover, Pavir.Fa00434.1 encoded the Myb-like DNA-binding domain. This gene was confirmed to control many biological functions like biotic and abiotic stress, development, defense, etc. in many organisms (Ambawat et al., 2013). In rice, the *MYB* gene controls Jasmonic acid (JA) against rice blast fungus: *Pyricularia greisea* (Lee et al., 2001). In Arabidopsis interaction with necrotrophic *Alternaria brassicicola*, the response to JA was the upregulated plant defensin protein (*Pdf*) gene. This gene was negatively controlled by the heat stress transcription factor B-1 encoded from Pavir.Fa00437.1 (Kumar et al., 2009). The most

interesting region linked with significant markers was on chromosome 7b since Pavir.Gb01488.1 and Pavir.Gb01488.2 were the only genes that overlapped with the BLAST result of the resistance to BLS in rice. These two genes encoded for cytochromes P450, which involved diverse oxidation reactions and triterpene synthesis (Geisler et al.,). The most well-known triterpene against fungal disease was Avenacins in oats (*Avena* spp.). Also, Pavir.Gb01298.1 encoded for the polyadenylate-binding protein. Polyadenylation is an important process to mediate transposon slicing of the RPP7 gene in Arabidopsis (Tsuchiya and Eulgem, 2013). Pavir.Gb01299.1 encoded for Isocitrate lyase, which was naturally a component of the glyoxylate cycle and was required for the pathogenicity of ascomycete *Leptosphaeria maculans,* causing blackleg disease of canola (*Brassica napus*) (Idnurm and Howlett, 2002). On chromosome 9a, the most interesting gene was Pavir.Ia00351.1 encoding mitogen-activated kinase (MAP). The mediation of the MAP has confirmed the activation of expression of ethylene (ET), JA, and salicylic acid (SA) in the response to both abiotic and biotic stress in Arabidopsis (Brader et al., 2007). Moreover, Pavir.Ia00174.1 encoded trichome Birefringence-like protein responsible for xylan acetylation in rice, which conferred the resistance to bacterial leaf blight (Gao et al., 2017). On chromosome 9b, Pavir.Ib01315.1, Pavir.Ib01315.2, Pavir.Ib01317.1 encoded glutathione S-transferase (GST) which was an important enzyme in redox reactions in cells. Glutathione was the key compound in signaling process to trigger plant resistance to pathogens (Dubreuil-Maurizi and Poinssot, 2012).

In 4X-MNY, on chromosome 5a, Pavir.Ea02827.1, encoding Actinidain, cysteine protease and Cathepsin propeptide inhibitor, was directly involved in

programmed cell death in the plant (Solomon et al., 1999). Additionally, Pavir.Ea02828.1 encoded DEAD/DEAH box helicase, which showed the up-regulation of defense-related signals like SA and JA and conferred the resistance again rice blast fungus *Manaporthe grisea* (Li et al., 2008). On chromosome 8a, Pavir.Ha00272.1 encoded for auxilin/cyclin G-associated kinase (GAK). Besides relating to kinase signal group conferring innate immunity, auxilin-like protein itself was confirmed to induce necrotic lesion and confer resistance to *Xanthomonas oryzae* pv. Oryzae (Xoo) (Park et al., 2017).

In low-DTIA-DSIA-MNY-MPA, on chromosome 1b, Pavir.Ab01193.1, Pavir.Ab01193.2, and Pavir.Ab01193.3 encoded ginkbilobin-2. This protein was purified from *Ginkgo biloba* seeds. It is cysteine-rich receptor-like kinase that confirmed the functions of salt stress and antifungal activity (Sawano et al., 2007; Miyakawa et al., 2009). On chromosome 3b, Pavir.Ca02197.1 encodedf ATP-binding cassette (ABC) transporter. There are many subgroups of ABC. In Arabidopsis, the mutant lacking the ABC transporter of PENETRATION3/ PLEIOTROPIC DRUG RESISTANCE8 (PEN3/PDR8) conferred nonhost susceptibility to *Blumeria graminis* f. sp hordei., suggesting that the ABC transporter was involved in exporting secreted toxins from the fungus and fungal suppressor from the host (Stein et al., 2006). Also, in wheat, the ABC suggested the exportation of mycotoxin conferring resistance to Fusarium head blight (Walter et al., 2015). On chromosome 3b, Pavir.Cb01412.1 encoded copines, which were diverse protein classes related to calcium-, phospholipid-binding function. The study in Arabidopsis suggested that copines acted as suppressors of defense responses in hypersensitive cell death defense against

*Pseudomonas syringae* pv *tomato* (Jambunathan and McNellis, 2003). On chromosome 5b, Pavir.Eb00172.1 encoded serine/threonine protein kinase (STPK) which were known to be candidates conferring disease resistance in wheat to tan spot (Juliana et al., 2018) and Arabidopsis to flagellin bacteria (Gómez-Gómez and Boller, 2000). Pavir.Eb00173.1 encoded BED zing finger which was classed as NBS-LRR, which played an important role in effector-triggered immunity (ETI). The zing finger domains suggested the broad-spectrum nature of rice blast resistance gene *Pi54* (Gupta et al., 2012). Pavir.Eb00177.1 encoded chloroplastic membrane protein, which also can involve mediating ROS from avirulent pathogen invasion (Yaeno et al., 2004).

In up-DTVI-MNY, although there was only one significant peak of markers on chromosome 7, it was unlikely to conclude that there was an R gene in this chromosome due to multiple significant SNPs with diverse roles. Pavir.Ga00855.1 encoded STPK similarly to Pavir.Eb00172.1 on chromosome 5. Pavir.Ga00857.1 and Pavir.Ga00858.1encoded for transporter proteins like Pavir.Ca02197.1 on chromosome 3b. Lastly, Pavir.Ga00912.1 encoded WD40 repeat family protein. Although there was no study directly with disease resistance, WD40 is involved in many biosynthesis pathways such as Transparent Testa2, which is a Myb transcription factor related to the resistance as explained earlier, and flavonoid biosynthesis for antioxidation handling ROS.

Although these candidate genes from the switchgrass association panel shared some of the functions from candidate genes from rice resistance to BLS, some of the potential functions conferring resistance were still missing such as dirigent protein, peroxidase, and Mlo-like protein. Dirigent protein conferred resistance by lignin and

lignan synthesis (Ralph et al., 2006). The lignin synthesis was expected to relate with resistance to BLS by strengthening cell walls (Dallagnol et al., 2013). However, since none of the resistance traits for BLS correlated with lignin, the missing dirigent protein from detection can be expected. Peroxidase also played an important role in Pathogen-associated molecular pattern-triggered immunity (PTI) (Mammarella et al., 2015). This was unexpected due to the high involvement of ROS in the infection of BO (Antonious and Jawhar, 2013). Mlo was identified to provide broad resistance to powdery mildew in barley (Jorgensen, 1992). More powerful model and finer phenotype combinations were needed to clarify these missing candidates.

In conclusion, the resistance to BLS yielded differently based on different phenotyping approaches, with low correlations suggesting that resistance was sensitive to phenotyping methods in different conditions. Although it is difficult to standardize the phenotyping approach that can take account all conditions, finer and more powerful phenotyping of the resistance is still needed. As such, to the best of our phenotyping combination, SW805 should be considered the most resistant population. Based on obtained phenotypes, our multi-trait GWAS with four subsets from 479 genotypes have a handful of markers that can be considered as potential candidate genes related to resistance to BLS with reasonable biological functions. Nevertheless, one needs to be prudent regarding the utilization of these markers. Due to low correlations between resistance to BLS from different phenotyping approaches, the combinations of the trait in a different subgroup of genotypes provided no significant overlapping regions. The application of these markers cannot be directly transferred

for genomics-assisted selection without validation to the choices of phenotyping approaches in new populations.

When considering biological functions from the GWAS regardless of subgroup and trait combinations, the defense mechanisms against BLS are complicated and multifaceted, probably controlled by multiple small-effect alleles. Most of the responses involved ETI via signaling kinases and NBS-LRR. Moreover, basal defense mechanism and PTI mostly via oxidative stress also played an important role in response to BLS. Despite missing expected candidate functions and only one overlapping gene for resistance to BLS in rice, most of the genes from these GWASs in switchgrass encode proteins overlapping with genes in rice. To the best of my knowledge, this was first research to dissect the resistance to BLS in switchgrass. With the current power of detection within the population, multi-trait GWAS can provide functional insight into the resistance within multiple subsets of genotypes.

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# CHAPTER 5

## EFFECTS OF SILICON AMENDMENT ON RESISTANCE TO BIPOLARIS DISEASES IN SWITCHGRASS


### Abstract

High yield with relatively low input to keep price competitiveness are the key characters of a reliable biomass crop. North America native perenniel switchgrass (*Panicum virgatum* L.) is a promising candidate for a biomass crop. To maintain reliable yield of switchgrass for biomass production, decreasing the effect of yield-reducing diseases is a must. One of the pathogenic fungi in switchgrass is *Bipolaris oryzae* (Breda de Haan) Shoemaker (BO) causing Bipolaris seed rot (BSR) and Bipolaris leaf spot (BLS). The diseases reduce biomass and seed germination. In Bipolaris disease management, silicon amendment showed potential features including persistence of silicon in the field, the triggered plant immunity against BO, and existing silicon protein transporters in switchgrass (Lsi1, Lsi2, and Lsi6). Thus, the effects of silicon on resistance to BSR and BLS were tested by mixing silicon with Cornell Peat-lite media and by watering silicon solution during seedling inoculation in 'Cave-in-Rock', 'Kanlow', 'Blackwell', and 'Shelter'. Germination, post-emergent BLS, and percentage of leaf covered with lesions from BSR and BLS were collected. The results showed no effects of silicon application to either BSR or BLS. This can potentially be explained by 1) the low retention of silicon in Cornell Peat-lite media, 2) no biological effect of silicon on the defense mechanism in seed to BSR, and 3) the stage of seedling that cannot accumulate enough silicon. This research cannot infer the effect of the silicon in a field trial and longer effects on diseases in mature plants.

## Introduction

Alternative renewable energy is explored for a sustainable resource. Biofuel from biomass is considered one of the prominent resources. The US Department of Energy has suggested switchgrass (*Panicum virgatum* L.) as a potential crop for biomass production (McLaughlin and Walsh, 1998). The advantages of switchgrass over other biomass crops are high biomass yield, low input, and well-adaptation in poor land (Sanderson et al., 2006; Schmer et al., 2008; Lee et al., 2009). As a biomass crop, the highest priority of the crop is to maintain high yield with low input to keep its economic competitiveness. However, switchgrass yield can be suffered from diseases.

*Bipolaris oryzae* (Breda de Haan) Shoemaker (BO) is one of the fungi damaging yield and field establishment of switchgrass (Fajolu et al., 2012). The fungus can cause two different diseases – Bipolaris seed rot (BSR) and Bipolaris leaf spot (BLS). These diseases can reduce yield up to 50% and seed germination up to 75% (Fajolu et al., 2012). As a soil-borne fungus, BO can colonize in soil and crop residue saprophytically (Ou, 1985). Being a seed-borne fungus, BO can be transmitted via infected seed contamination and is considered as a primary inoculation to the field. Both resources can later provide the inoculation to seedlings and mature plants causing BLS.

Chemical application is one of approaches for Bipolaris disease management. Application of fungicides such as Bavistin, Hinosan, Tilt 250 EC (Propiconazole), and Dithane M-45 has tested the effectiveness against BO in rice (Ahmed et al., 2002). However, the fungicide needed to be applied annually to keep the effectiveness, resulting in less price competitiveness. An alternative chemical was silicon amendment.

Since silicon residual can persist in soil over time, annual reapplication is not required (Alvarez and Datnoff, 2001). In rice silicon improved resistance to BLS (Bockhaven et al., 2015). The accumulation of silicon on the epidermis physically prevents the growth and penetration of BO. Moreover, the silicon also triggers defense signals including salicylic acid and jasmonic acid. Interestingly, silicon prevents BO from taking over rice ethylene production. Since the silicon transporter, Lsi1 and Lsi2 were identified in tetraploid switchgrass (Palmer et al., 2014), this defense mechanism was expected to occur in switchgrass. However, the response to silicon amendment to BSR and BLS in switchgrass have never been reported.

Thus, the objective of this study was to investigate the effects of silicon amendment on the resistance to BLS and BSR.

## Materials and Methods

### Bipolaris isolate source and inoculum preparation

The spore isolate of *B. oryzae* Bo008NY07 was isolated from a 'Carthage' switchgrass leaf in the warm season biofuels field experiment in Ithaca, NY, by Katie Waxman in 2007 (Waxman and Bergstrom, 2011). The spore isolate of *Bipolaris oryzae* was subcultured on one-strength potato dextrose agar (PDA). To prepare inoculum, on the day of inoculation, three-week-old *B. oryzae* in petri dishes were flooded with sterile deionized water and scraped with a glass spreader. The suspension of conidia was filtered via gauze to remove residue of the PDA and mycelium. The suspension was then adjusted to a concentration of conidia by hemocytometer under a microscope. Two drops of Tween-20 were added to 100 mL of inoculum as a surfactant.

**Effects of silicon amendment on BSR and BLS**

All silicon amendment trials were conducted in a greenhouse under a 12-hr photoperiod of supplemental light by high-pressure sodium lamps at 40 klm and ambient temperatures of 18 to 29°C. The test of silicon effects was separated into two silicon application methods – mixing in media and watering with silicon solution. In both methods, the seed inoculum was done in the ratio of 0.2 g seed/25 mL inoculum at 60 rpm shaking condition for 10 minutes. The inoculum residue was filtered out of the inoculated seeds, and the seeds were dried overnight before planting in 288-cell trays.

***Mixing media with silicon solution***

To reach the maximum silicon incorporation rate in media at 200 $mg \cdot L^{-1}$, one liter of Cornell Peat-lite media was mixed with 810.31 mg of Agsil 16H (24.7% silicon in the powder). The control media was mixed with $K_2SO_4$ to compensate the $K_2O$ in the silicon powder at 485.58 $mg \cdot L^{-1}$ of media. The control and silicon mixed media was filled in four 288-cell trays. The left half was filled with control mixed media and the right half was filled with silicon mixed media. The seeds 'Cave-in-Rock' (CIR) and 'Kanlow' (KL) were inoculated with 0, $10^5$ $conidia \cdot mL^{-1}$ and planted in random rows in each tray. The trays were kept in a greenhouse and watered normally at twice a day. After four weeks, the germinating seedlings were inoculated with conidia suspension at 0, and $10^5$ $conidia \cdot mL^{-1}$ by an airbrush at 20 psi, covered with a clear plastic bag for 24 hours, kept in the greenhouse for 7 days, and evaluated for BLS severity as percentage of leaf covered by lesions (PLC).

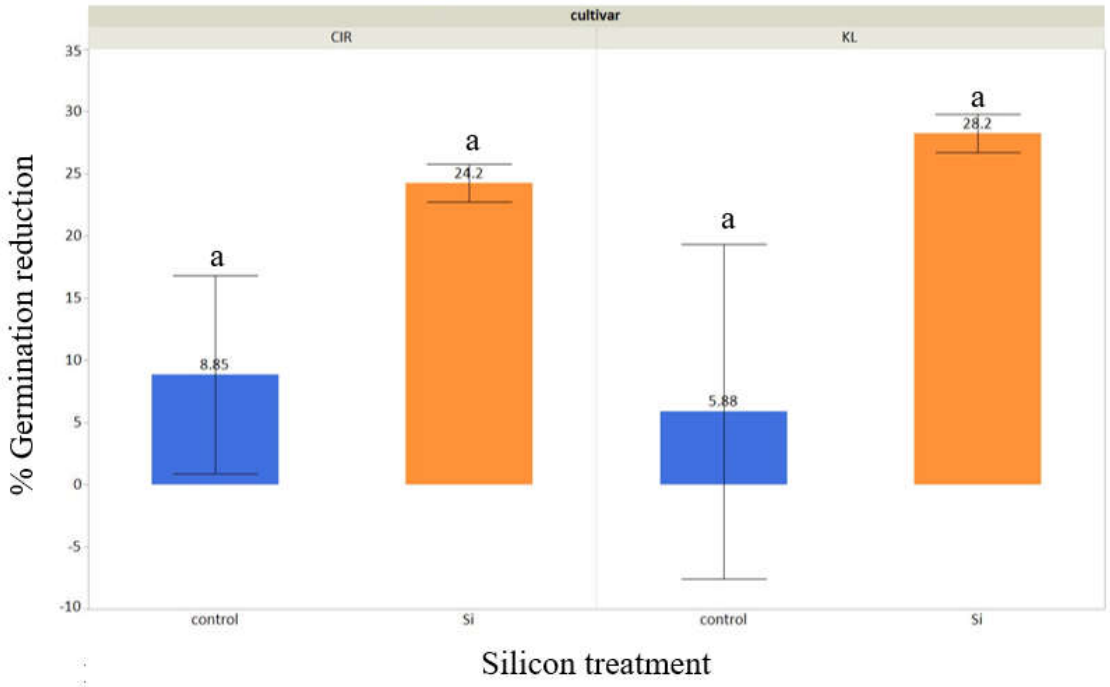
### *Watering with silicon solution*

Seeds of CIR, 'Blackwell' (BW), and 'Shelter' (ST) were surface-sterilized with 95% ethanol for 1 min, 10% bleach for 1 min, rinsed with de-ionized water three times, and dried overnight. The inoculum concentrations were 0 as a control, $10^3$, $10^4$ and $10^5$ conidia·mL$^{-1}$. Twelve entries of cultivars×seed inoculations were randomly planted as two seeds in each of 24 of 7-cm$^3$ cells in 288-cell trays filled with Cornell Peat-lite media. After three weeks, silicon treatments were applied to each tray daily for two weeks. Each tray was divided in half. The right half was watered with 2 ml Si of 100 mg·L$^{-1}$Dyna-Gro Pro-TeKt (7.8% silicon) per cell (156 mg Si/cell), and the left half was watered with 71.14 mg/L of $K_2SO_4$ as a control to compensate for K in the Dyna-Gro treatment. At the fourth week, numbers of emerged and uninfected seedling numbers were recorded, and severity of leaf spot was scored based on a percentage of leaf area with necrotic lesions on the two top leaves to determine the control of Bipolaris seed rot. The remaining seedlings not showing foliar necrosis resulting from seed inoculations were then subjected to foliar inoculation. Five replicated trays were sprayed with 22 ml of each of four concentrations of conidial suspensions by an airbrush pressurized at 20 psi and covered with clear plastic bags for 24 hrs. Percent necrosis on the two top leaves was recorded at seven days post inoculation to determine the control of Bipolaris leaf spot.

Statistical analysis was performed by analysis of variance, and the Tukey-Kramer HSD test ($P = 0.05$) was used for mean separation comparison between treatments.

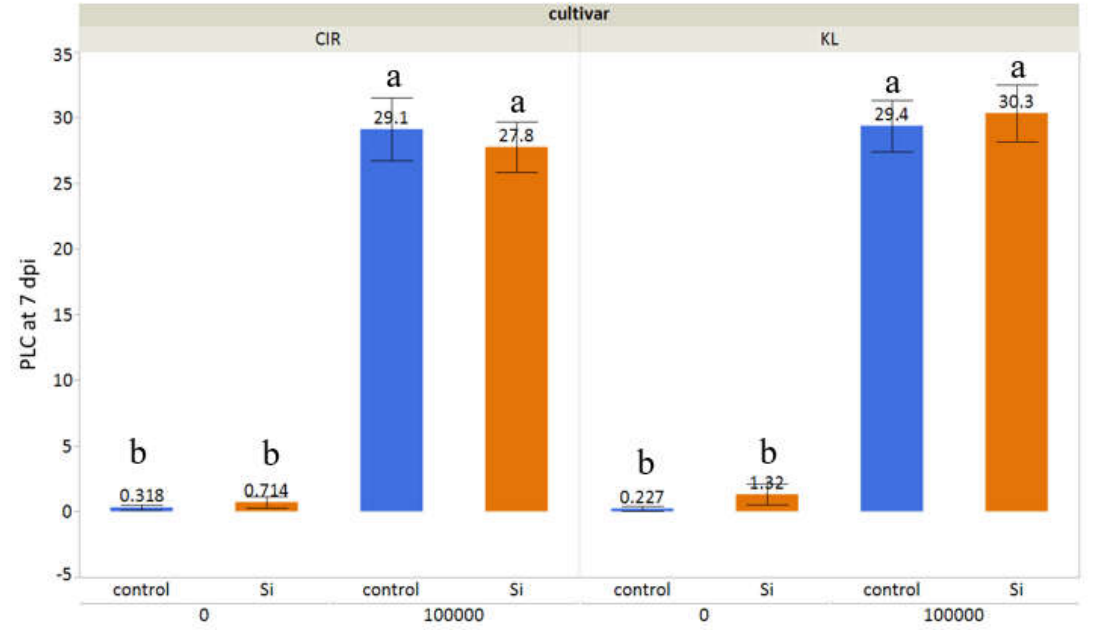
**Results**

The silicon application via media mixing showed no significant effect on BSR in both CIR and KL (Figure 5.1). Unexpectedly, the silicon application showed a pattern of greater germination reduction, however this pattern was not statistically significant. The lack of improvement of resistance to BLS by silicon application via media mixing was confirmed from the non-significantly different PLC between control and silicon treatment (Figure 5.2).




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 5.1 Effect of silicon application in mixed Cornell Peat-lite medium on BSR as in reduction percentages of germination of inoculated seeds compared with control seeds in CIR and KL.




Different letters represent significantly different groups from Tukey's HSD at $\alpha = 0.05$

Figure 5.2 Effect of silicon application in soil medium on BLS as PLC at 7 dpi in CIR and KL.

The other approach of silicon amendments via watering silicon solution also showed no effects in reducing the diseases in switchgrass (Table 5.1). Control of BSR was estimated by a lesser reduction in germination of silicon-treated plants than of control plants that were seed-inoculated with Bipolaris and by a reduction in foliar disease severity post-germination. Both estimates showed some evidence for improvement in control with silicon in Blackwell in response to all concentrations of conidia; however, only the leaf spot at $10^5$ conidia·$mL^{-1}$ treatment was significantly different between the silicon amendment and the control treatment. The other treatments remained the same in the other cultivars. Similarly, control of BLS represented by percent foliar necrosis resulting from foliar inoculation showed no improvement from silicon amendment in any treatments.

Table 5.1 Effects of watering silicon solution for two weeks before seedling inoculation on BSR and BLS in BL, CIR, and ST at various concentrations of inoculum.

| Cultivars | Inoculum (100 conidia·mL-1) | Silicon treatment | Bipolaris seed rot | | | | Bipolaris leaf spot | |
|---|---|---|---|---|---|---|---|---|
| | | | Reduction in germination (%) | | Severity (%) | | Severity (%) | |
| Blackwell | 0 | non-treated | - | | 5.2 | ab | 0.1 | a |
| | | Dyna-Gro Pro-TeKt | - | | 5.2 | ab | 0.2 | a |
| | 10 | non-treated | 2.8 | ab | 5.1 | ab | 10.1 | a |
| | | Dyna-Gro Pro-TeKt | 1.5 | a | 4.9 | ab | 18.8 | ab |
| | 100 | non-treated | 30.0 | abc | 6.2 | ab | 35.3 | c |
| | | Dyna-Gro Pro-TeKt | 8.7 | ab | 4.2 | b | 36.6 | c |
| | 1000 | non-treated | 23.4 | abc | 12.3 | a | 56.1 | d |
| | | Dyna-Gro Pro-TeKt | 15.4 | abc | 3.6 | b | 58.4 | d |
| Cave-in-Rock | 0 | non-treated | - | | 5.4 | ab | 0.1 | a |
| | | Dyna-Gro Pro-TeKt | - | | 0.9 | b | 0.1 | a |
| | 10 | non-treated | 6.6 | ab | 4.4 | b | 11.5 | a |
| | | Dyna-Gro Pro-TeKt | -3.8 | a | 2.6 | b | 9.8 | a |
| | 100 | non-treated | 7.6 | ab | 4.1 | b | 31.6 | bc |
| | | Dyna-Gro Pro-TeKt | 14.6 | abc | 4.0 | b | 33.2 | bc |
| | 1000 | non-treated | 23.3 | abc | 7.0 | ab | 62.0 | d |
| | | Dyna-Gro Pro-TeKt | 29.3 | abc | 5.4 | ab | 62.3 | d |
| Shelter | 0 | non-treated | - | | 1.7 | b | 0 | a |
| | | Dyna-Gro Pro-TeKt | - | | 0.6 | b | 0 | a |
| | 10 | non-treated | 3.3 | ab | 1.3 | b | 8.0 | a |
| | | Dyna-Gro Pro-TeKt | 4.5 | ab | 0.9 | b | 5.3 | a |
| | 100 | non-treated | 32.4 | abc | 6.0 | ab | 27.1 | bc |
| | | Dyna-Gro Pro-TeKt | 32.8 | abc | 3.5 | b | 29.1 | bc |
| | 1000 | non-treated | 40.8 | bc | 4.5 | ab | 65.9 | d |
| | | Dyna-Gro Pro-TeKt | 47.8 | c | 3.2 | b | 64.0 | d |
| HSD ($P = 0.05$) | | | 37.5 | | 7.9 | | 13.8 | |
| CV (%) | | | 174.4 | | 442.3 | | 53.5 | |

**Discussion**

The silicon amendment showed no significant effects on improving resistance to the diseases with the exception of Blackwell at $10^5$ conidia·$mL^{-1}$. The seed inoculation for screening for BSR might not get the benefit of silicon application since the effectiveness of silicon was shown in silicon translocation from root to shoot for a physical barrier (Bockhaven et al., 2015). The silicon could even provide a negative result to seed germination as the silicon treatment in mixing media showed higher reduction in germination. The increase in germination reduction could come from the increase of pH from silicon application over the optimum range of pH 5 to 8 (Hanson and Johnson, 2005). In BLS, despite the expected silicon transport pathway in switchgrass, the silicon amendments did not provide any benefits to the resistance. The Cornell Peat-lite media mixed with silicon could potentially lose the silicon through leaching from the daily watering for four weeks before the seedlings were inoculated. In spite of watering silicon daily for two weeks before and after seedling inoculation to reduce leaching of the silicon, the seedlings showed no improvement to BLS. This may suggest that the seedling between 3 to 5 weeks old cannot accumulate not enough silicon. Although the silicon application in this experiment did not result in significant disease control, the experiment was conducted in a greenhouse and may not reflect field conditions. Since the silicon was applied to soilless media, this may also influence silicon retention. We recommend that experiments be conducted to assess the effects of silicon on older seedlings in hydroponic culture and that silicon amendment also be assessed in longer-term studies in the field.

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# APPENDIX I

## RECURRENT PHENOTYPIC SELECTION

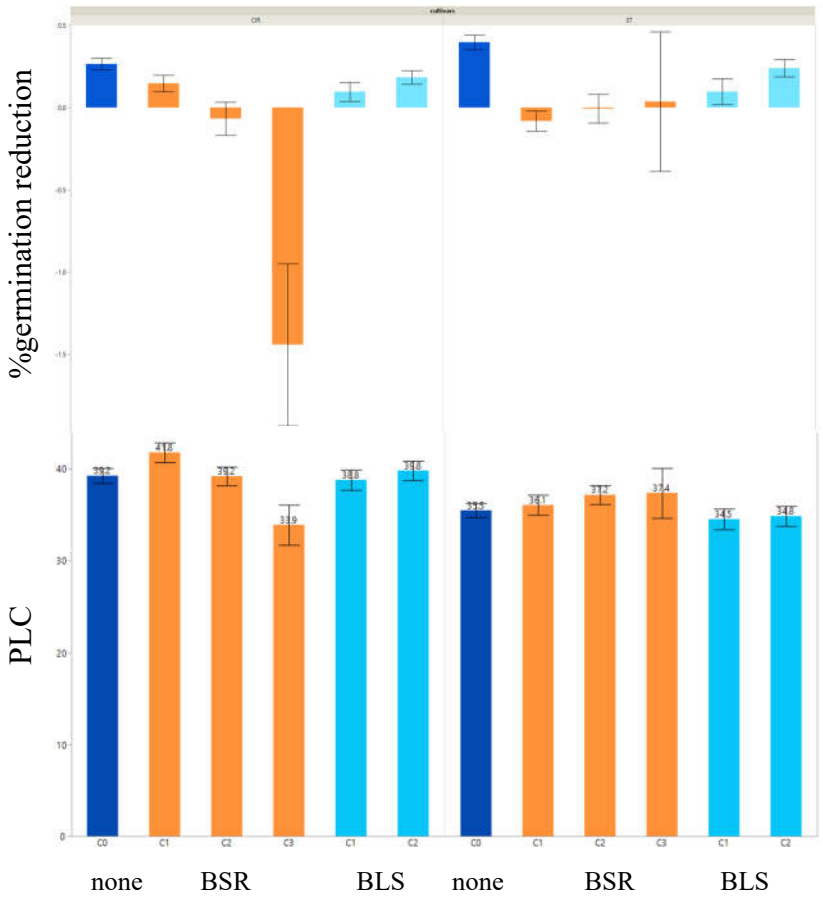


Figure I.1 Comparison between progresses of selection for resistance to BSR in germination reduction percentage (orange) for 3 cycles of selection and BLS in PLC (light blue) for 2 cycles of selection in CIR (left) and ST (right).

The third cycle of selection for resistance to BSR was excluded from the research due to inconsistency of the result with the other cycles. The germinations of control seeds from cycle 3 in both cultivars were significantly lower than the previous years and inoculated seeds show high variation in germination. This can be explained from the outlier of high humidity in 2017 when cycle 3 was planted in the field. Such a high humidity is favorable to *Bipolaris oryzae*.

## APPENDIX II

## GENOME-WIDE ASSOCIATION

Table II.1 Percentage of lesion on detached leaves from leaf detachment assay from 85 genotypes in three experiment sets. The phenotype was assessed via vision and image analysis.

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| Blackwell_04 | 1 | 10 (26) | 26.67 (133) | 13.33 (69) | 8.08 (134) | 10.94 (179) | 1.63 (29) |
| Blackwell_04 | 2 | 10 (26) | 10 (30) | 10 (36) | 5.27 (82) | 2.73 (55) | 1.78 (38) |
| Blackwell_04 | 3 | 20 (109) | 10 (30) | 13.33 (69) | 5.22 (80) | 3.2 (70) | 1.96 (52) |
| Blackwell_06 | 1 | 43.33 (193) | 23.33 (119) | 26.67 (150) | 8.84 (144) | 3.22 (68) | 3.97 (124) |
| Blackwell_06 | 2 | 43.33 (193) | 20 (97) | 10 (36) | 8.81 (142) | 4.83 (99) | 2.47 (65) |
| Blackwell_06 | 3 | 53.33 (222) | 16.67 (84) | 30 (167) | 11.06 (173) | 2.73 (54) | 8.22 (199) |
| Carthage_02 | 1 | 26.67 (143) | 40 (174) | 16.67 (86) | 11.34 (177) | 7.98 (146) | 3.07 (88) |
| Carthage_02 | 2 | 43.33 (193) | 20 (97) | 23.33 (131) | 12.89 (192) | 2.21 (31) | 2.75 (81) |
| Carthage_02 | 3 | 50 (209) | 30 (141) | 26.67 (148) | 21.34 (220) | 2.51 (44) | 7.63 (190) |
| Carthage_05 | 1 | 6.67 (8) | 16.67 (84) | 10 (36) | 6.78 (110) | 1.74 (15) | 5.56 (164) |
| Carthage_05 | 2 | 6.67 (8) | 3.33 (2) | 6.67 (18) | 7.14 (116) | 2.07 (24) | 4.21 (131) |
| Carthage_05 | 3 | 6.67 (8) | 16.67 (83) | 16.67 (84) | 4.64 (71) | 3.6 (72) | 7.44 (183) |
| Cave.in.Rock_04 | 1 | 60 (219) | 26.67 (124) | 36.67 (178) | 21.76 (217) | 14.46 (190) | 4.14 (127) |
| Cave.in.Rock_04 | 2 | 66.67 (226) | 73.33 (227) | 33.33 (169) | 28.02 (224) | 30.73 (225) | 5.33 (152) |
| Cave.in.Rock_04 | 3 | 90 (241) | 33.33 (148) | 36.67 (178) | 23.74 (220) | 5.72 (111) | 5.47 (161) |
| Cave.in.Rock_05 | 1 | 10 (23) | 30 (137) | 40 (183) | 0.77 (4) | 13.66 (183) | 26.02 (227) |
| Cave.in.Rock_05 | 2 | 23.33 (122) | 30 (137) | 6.67 (18) | 11.56 (176) | 11.24 (169) | 3.3 (100) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| Cave.in.Rock_05 | 3 | 46.67 (195) | 30 (137) | 50 (199) | 36.12 (223) | 11.27 (169) | 21.62 (220) |
| ECS.10_04 | 1 | 23.33 (121) | 56.67 (195) | 53.33 (201) | 10.26 (160) | 12.25 (175) | 14.42 (211) |
| ECS.10_04 | 2 | 26.67 (138) | 40 (166) | 30 (158) | 5.64 (88) | 8.32 (144) | 5.41 (155) |
| ECS.10_04 | 3 | 46.67 (193) | 26.67 (124) | 20 (102) | 7.75 (122) | 5.54 (109) | 6.44 (171) |
| ECS.12_05 | 1 | 10 (23) | 30 (134) | 60 (210) | 8.84 (136) | 8.89 (147) | 8.61 (186) |
| ECS.12_05 | 2 | 16.67 (85) | 16.67 (82) | 63.33 (215) | 8.4 (130) | 3.66 (72) | 10.64 (196) |
| ECS.12_05 | 3 | 43.33 (185) | 10 (29) | 86.67 (231) | 10.48 (160) | 2.57 (44) | 29.21 (225) |
| ECS.12_06 | 1 | 23.33 (119) | 43.33 (168) | 20 (102) | 5.61 (86) | 14.24 (177) | 6.19 (167) |
| ECS.12_06 | 2 | 30 (150) | 10 (29) | 20 (102) | 6.16 (96) | 5.75 (109) | 6.15 (167) |
| ECS.12_06 | 3 | 46.67 (189) | 26.67 (121) | 30 (156) | 5.2 (77) | 9.71 (154) | 10.98 (197) |
| ECS.6_01 | 1 | 10 (23) | 6.67 (11) | 3.33 (5) | 5.93 (89) | 2.73 (48) | 3.4 (103) |
| ECS.6_01 | 2 | 23.33 (118) | 23.33 (109) | 6.67 (17) | 12.11 (168) | 6.08 (111) | 4.39 (135) |
| ECS.6_01 | 3 | 46.67 (187) | 10 (28) | 10 (33) | 27.9 (209) | 3.38 (64) | 4.72 (141) |
| ECS.6_02 | 1 | 3.33 (3) | 50 (175) | 10 (32) | 6.26 (95) | 8.39 (137) | 2.97 (85) |
| ECS.6_02 | 2 | 10 (22) | 66.67 (201) | 10 (32) | 6.7 (101) | 20.04 (192) | 3.61 (107) |
| ECS.6_02 | 3 | 26.67 (130) | 56.67 (184) | 10 (32) | 14.21 (181) | 10.49 (154) | 1.79 (38) |
| ECS.6_03 | 1 | 16.67 (81) | 13.33 (51) | 23.33 (117) | 15.61 (187) | 4.18 (82) | 2.77 (81) |
| ECS.6_03 | 2 | 20 (98) | 16.67 (78) | 26.67 (133) | 9.85 (144) | 3.7 (70) | 6.53 (162) |
| ECS.6_03 | 3 | 23.33 (114) | 20 (87) | 30 (147) | 12.19 (165) | 4.52 (85) | 3.62 (106) |
| High.Tide_04 | 1 | 10 (22) | 43.33 (162) | 16.67 (77) | 8.2 (120) | 24.53 (196) | 4.2 (122) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| High.Tide_04 | 2 | 20 (97) | 33.33 (132) | 10 (32) | 18.9 (191) | 14.59 (171) | 3.2 (90) |
| High.Tide_04 | 3 | 23.33 (112) | 13.33 (51) | 20 (94) | 15.45 (183) | 4.4 (82) | 2.8 (81) |
| High.Tide_05 | 1 | 0 (1) | 20 (86) | 13.33 (57) | 0.38 (1) | 3.18 (61) | 4.71 (129) |
| High.Tide_05 | 2 | 6.67 (7) | 20 (86) | 13.33 (57) | 2.39 (19) | 3.36 (63) | 2.36 (61) |
| High.Tide_05 | 3 | 16.67 (80) | 30 (125) | 16.67 (76) | 8.04 (119) | 9.53 (142) | 4.14 (118) |
| High.Tide_06 | 1 | 30 (135) | 3.33 (2) | 10 (32) | 7.71 (113) | 1.4 (6) | 1.26 (19) |
| High.Tide_06 | 2 | 30 (135) | 13.33 (50) | 13.33 (56) | 4.59 (67) | 2.81 (48) | 1.83 (39) |
| High.Tide_06 | 3 | 33.33 (142) | 10 (27) | 10 (32) | 4.52 (66) | 2.06 (22) | 2.06 (50) |
| High.Tide_08 | 1 | 23.33 (109) | 23.33 (98) | 53.33 (178) | 2.82 (28) | 1.33 (4) | 9.3 (167) |
| High.Tide_08 | 2 | 43.33 (164) | 13.33 (49) | 40 (160) | 7.79 (111) | 1.21 (4) | 5.68 (142) |
| High.Tide_08 | 3 | 70 (194) | 16.67 (73) | 53.33 (178) | 7.88 (112) | 1.58 (9) | 5.35 (136) |
| Kanlow_05 | 1 | 23.33 (109) | 40 (143) | 23.33 (107) | 7.67 (107) | 9.16 (131) | 6.01 (145) |
| Kanlow_05 | 2 | 26.67 (120) | 50 (160) | 16.67 (71) | 6.95 (97) | 8.88 (129) | 8.24 (163) |
| Kanlow_05 | 3 | 43.33 (161) | 50 (159) | 16.67 (71) | 7.61 (104) | 10.66 (139) | 2.32 (57) |
| KY1625_04 | 1 | 13.33 (54) | 53.33 (160) | 50 (171) | 3.5 (39) | 19.91 (171) | 17.1 (181) |
| KY1625_04 | 2 | 26.67 (119) | 40 (143) | 26.67 (121) | 8.23 (108) | 16.73 (165) | 6.72 (149) |
| KY1625_04 | 3 | 53.33 (178) | 43.33 (149) | 50 (171) | 18.4 (175) | 15.43 (161) | 13.71 (177) |
| KY1625_05 | 1 | 6.67 (6) | 3.33 (2) | 16.67 (69) | 3.22 (37) | 1.84 (13) | 2.66 (67) |
| KY1625_05 | 2 | 10 (20) | 10 (27) | 13.33 (55) | 5.25 (71) | 5.35 (88) | 1.45 (23) |
| KY1625_05 | 3 | 40 (151) | 13.33 (49) | 13.33 (55) | 12.34 (152) | 2.54 (38) | 1.53 (27) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| Pathfinder_07 | 1 | 73.33 (185) | 63.33 (171) | 13.33 (55) | 42.65 (190) | 22.06 (171) | 2.76 (72) |
| Pathfinder_07 | 2 | 83.33 (194) | 46.67 (149) | 23.33 (102) | 64.89 (196) | 20.97 (169) | 4.29 (113) |
| Pathfinder_07 | 3 | 90 (196) | 36.67 (132) | 6.67 (17) | 56.91 (195) | 11.33 (138) | 3.09 (76) |
| Shelter_06 | 1 | 50 (165) | 40 (138) | 36.67 (142) | 22.22 (175) | 5.43 (87) | 4.17 (107) |
| Shelter_06 | 2 | 56.67 (176) | 63.33 (168) | 23.33 (100) | 11.24 (140) | 17.77 (161) | 4.27 (110) |
| Shelter_06 | 3 | 80 (191) | 10 (26) | 16.67 (67) | 19.91 (174) | 0.88 (1) | 3.09 (75) |
| Shelter_07 | 1 | 10 (19) | 36.67 (130) | 26.67 (111) | 2.42 (18) | 7.4 (107) | 2.31 (54) |
| Shelter_07 | 2 | 20 (91) | 36.67 (131) | 13.33 (54) | 4.47 (61) | 7.32 (106) | 3.99 (101) |
| Shelter_07 | 3 | 20 (91) | 10 (26) | 10 (31) | 3.06 (32) | 4.11 (69) | 4.12 (105) |
| Shelter_08 | 1 | 10 (19) | 66.67 (167) | 33.33 (130) | 1.03 (3) | 16.17 (153) | 4.79 (114) |
| Shelter_08 | 2 | 10 (19) | 73.33 (173) | 23.33 (98) | 1.17 (3) | 20.22 (160) | 3.45 (87) |
| Shelter_08 | 3 | 13.33 (49) | 56.67 (153) | 53.33 (158) | 1.47 (6) | 17.36 (155) | 2.72 (70) |
| Shelter_09 | 1 | 33.33 (129) | 40 (135) | 23.33 (96) | 6.2 (80) | 5.14 (81) | 3.84 (94) |
| Shelter_09 | 2 | 60 (170) | 40 (135) | 10 (31) | 13.71 (151) | 6.9 (101) | 3.06 (72) |
| Shelter_09 | 3 | 60 (170) | 63.33 (162) | 20 (77) | 9.69 (116) | 14.45 (146) | 4.07 (99) |
| SW102_06 | 1 | 76.67 (176) | 96.67 (179) | 90 (183) | 52.4 (182) | 35.53 (170) | 42.71 (181) |
| SW102_06 | 2 | 76.67 (176) | 100 (181) | 80 (175) | 39.79 (177) | 70.5 (182) | 16.68 (162) |
| SW102_06 | 3 | 76.67 (176) | 96.67 (179) | 83.33 (176) | 35.48 (171) | 46.08 (177) | 19.76 (163) |
| SW110_01 | 1 | 23.33 (100) | 33.33 (116) | 10 (31) | 5.83 (73) | 10.04 (125) | 2.83 (70) |
| SW110_01 | 2 | 23.33 (100) | 26.67 (101) | 20 (76) | 8.38 (98) | 8.41 (115) | 4.38 (102) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW110_01 | 3 | 50 (158) | 26.67 (101) | 30 (119) | 23.4 (166) | 8.8 (118) | 5.34 (115) |
| SW110_04 | 1 | 16.67 (71) | 10 (26) | 60 (159) | 6.17 (78) | 4.86 (75) | 7.11 (129) |
| SW110_04 | 2 | 16.67 (71) | 20 (75) | 33.33 (123) | 9.31 (108) | 3.98 (66) | 4.64 (105) |
| SW110_04 | 3 | 16.67 (71) | 6.67 (9) | 36.67 (130) | 8.31 (96) | 2.13 (17) | 3.64 (88) |
| SW114_02 | 1 | 6.67 (6) | 13.33 (43) | 23.33 (94) | 7.07 (88) | 3.54 (55) | 5.34 (111) |
| SW114_02 | 2 | 13.33 (48) | 26.67 (97) | 16.67 (63) | 5.4 (69) | 7.51 (102) | 3.17 (73) |
| SW114_02 | 3 | 23.33 (95) | 23.33 (89) | 13.33 (51) | 10.27 (118) | 11.87 (128) | 1.92 (43) |
| SW116_01 | 1 | 36.67 (127) | 76.67 (161) | 56.67 (148) | 6.53 (81) | 40.5 (161) | 11.84 (144) |
| SW116_01 | 2 | 50 (150) | 73.33 (160) | 40 (131) | 11.49 (127) | 28.02 (155) | 5.65 (112) |
| SW116_01 | 3 | 60 (160) | 73.33 (160) | 46.67 (140) | 11.8 (129) | 33.06 (159) | 4.86 (103) |
| SW116_03 | 1 | 13.33 (48) | 6.67 (9) | 23.33 (92) | 7.65 (90) | 2.66 (38) | 3.12 (70) |
| SW116_03 | 2 | 20 (81) | 13.33 (42) | 23.33 (92) | 6.51 (80) | 2.91 (43) | 3.45 (81) |
| SW116_03 | 3 | 43.33 (138) | 20 (71) | 23.33 (92) | 17.22 (146) | 3.74 (57) | 3.8 (86) |
| SW122_06 | 2 | 10 (18) | 33.33 (106) | 73.33 (155) | 1.3 (3) | 9.65 (113) | 26.97 (156) |
| SW122_06 | 1 | 13.33 (48) | 33.33 (106) | 76.67 (158) | 5.02 (62) | 6.46 (88) | 30.02 (159) |
| SW122_06 | 3 | 16.67 (66) | 30 (101) | 63.33 (151) | 6.05 (73) | 3.45 (51) | 25.16 (155) |
| SW123_02 | 1 | 10 (18) | 30 (101) | 33.33 (117) | 1.32 (3) | 4.95 (70) | 6.28 (116) |
| SW123_02 | 2 | 16.67 (65) | 26.67 (93) | 20 (74) | 2.15 (9) | 3.64 (53) | 5.47 (108) |
| SW123_02 | 3 | 40 (126) | 26.67 (93) | 30 (110) | 2.01 (8) | 3.18 (47) | 3.29 (75) |
| SW123_10 | 1 | 16.67 (65) | 46.67 (122) | 36.67 (121) | 12.3 (119) | 10.96 (111) | 5.43 (105) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW123_10 | 2 | 26.67 (95) | 20 (71) | 60 (145) | 11.13 (114) | 3.22 (48) | 9.81 (129) |
| SW123_10 | 3 | 30 (105) | 16.67 (63) | 60 (144) | 9.59 (97) | 5.9 (76) | 12.85 (138) |
| SW127_06 | 1 | 10 (18) | 56.67 (129) | 6.67 (17) | 2.38 (10) | 7.66 (91) | 1.5 (23) |
| SW127_06 | 2 | 10 (18) | 43.33 (117) | 23.33 (89) | 3.79 (32) | 6.61 (83) | 4.49 (94) |
| SW127_06 | 3 | 16.67 (65) | 16.67 (63) | 10 (31) | 3.59 (31) | 1.19 (2) | 1.22 (18) |
| SW127_07 | 1 | 10 (18) | 26.67 (90) | 20 (72) | 2.5 (10) | 7.91 (93) | 4.51 (94) |
| SW127_07 | 2 | 16.67 (62) | 36.67 (107) | 33.33 (111) | 3.1 (23) | 10.2 (103) | 10.8 (128) |
| SW127_07 | 3 | 26.67 (90) | 6.67 (9) | 36.67 (117) | 3.75 (29) | 1.83 (10) | 11.61 (131) |
| SW33_03 | 1 | 3.33 (2) | 50 (119) | 36.67 (116) | 3.87 (30) | 6.4 (79) | 6.99 (110) |
| SW33_03 | 2 | 13.33 (42) | 46.67 (115) | 46.67 (125) | 4.93 (50) | 7.67 (87) | 20.45 (136) |
| SW33_03 | 3 | 20 (69) | 20 (68) | 73.33 (141) | 6.47 (66) | 2.4 (23) | 23.81 (139) |
| SW33_04 | 1 | 6.67 (5) | 50 (117) | 56.67 (133) | 7.19 (70) | 15.89 (119) | 9.53 (122) |
| SW33_04 | 2 | 10 (16) | 33.33 (96) | 43.33 (120) | 5.09 (50) | 11.82 (105) | 11.43 (126) |
| SW33_04 | 3 | 60 (136) | 36.67 (106) | 53.33 (130) | 37.42 (140) | 9.53 (97) | 5.91 (104) |
| SW38_04 | 1 | 16.67 (58) | 6.67 (9) | 46.67 (124) | 9.81 (88) | 5.22 (65) | 13.85 (127) |
| SW38_04 | 2 | 20 (66) | 10 (23) | 46.67 (124) | 9.72 (87) | 3.11 (42) | 7.57 (111) |
| SW38_04 | 3 | 30 (94) | 10 (23) | 56.67 (131) | 16.98 (125) | 5.74 (72) | 18.47 (131) |
| SW43_04 | 1 | 6.67 (5) | 76.67 (134) | 13.33 (49) | 1.57 (4) | 13.73 (107) | 4.09 (88) |
| SW43_04 | 2 | 10 (15) | 30 (90) | 3.33 (5) | 2 (6) | 7.41 (83) | 4.02 (86) |
| SW43_04 | 3 | 16.67 (56) | 66.67 (126) | 10 (29) | 4.5 (43) | 12.69 (104) | 5.05 (94) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW49_01 | 1 | 10 (15) | 26.67 (85) | 13.33 (47) | 4.28 (40) | 6.33 (73) | 10.02 (117) |
| SW49_01 | 2 | 13.33 (37) | 33.33 (91) | 16.67 (56) | 2.55 (8) | 16.33 (110) | 5.82 (100) |
| SW49_01 | 3 | 20 (60) | 23.33 (78) | 3.33 (5) | 11.13 (94) | 8.3 (86) | 3.56 (79) |
| SW50_05 | 1 | 36.67 (97) | 60 (117) | 30 (100) | 12.33 (99) | 29.9 (122) | 12.69 (120) |
| SW50_05 | 2 | 36.67 (97) | 53.33 (108) | 26.67 (88) | 10.1 (85) | 10.99 (94) | 10.16 (115) |
| SW50_05 | 3 | 40 (102) | 43.33 (104) | 33.33 (104) | 8.87 (75) | 31.32 (122) | 7.74 (109) |
| SW64_05 | 1 | 80 (129) | 90 (129) | 83.33 (127) | 42.75 (128) | 43.4 (125) | 29.75 (125) |
| SW64_05 | 2 | 86.67 (130) | 100 (130) | 86.67 (129) | 45.21 (128) | 62.19 (130) | 51.82 (130) |
| SW64_05 | 3 | 83.33 (131) | 60 (115) | 73.33 (125) | 66.65 (132) | 23.95 (117) | 28.11 (125) |
| SW65_07 | 1 | 6.67 (5) | 20 (65) | 10 (26) | 0.52 (1) | 5.39 (67) | 4.05 (84) |
| SW65_07 | 2 | 10 (15) | 30 (87) | 6.67 (14) | 0.67 (2) | 14.67 (104) | 4.88 (91) |
| SW65_07 | 3 | 23.33 (69) | 20 (65) | 3.33 (5) | 4.25 (37) | 6.03 (71) | 1.19 (16) |
| SW65_08 | 1 | 40 (98) | 26.67 (82) | 16.67 (52) | 7.65 (63) | 2.89 (39) | 1.84 (34) |
| SW65_08 | 2 | 46.67 (106) | 13.33 (38) | 16.67 (52) | 15.86 (108) | 2.88 (38) | 3.15 (66) |
| SW65_08 | 3 | 53.33 (115) | 16.67 (58) | 10 (26) | 25.55 (115) | 2.77 (35) | 2.51 (53) |
| SW781_05 | 1 | 23.33 (67) | 13.33 (38) | 16.67 (51) | 10.59 (84) | 6.37 (68) | 3.41 (73) |
| SW781_05 | 2 | 26.67 (72) | 13.33 (38) | 20 (59) | 8.97 (71) | 8.79 (81) | 2.7 (62) |
| SW781_05 | 3 | 43.33 (101) | 20 (62) | 20 (59) | 18.5 (109) | 12.29 (92) | 3.23 (65) |
| SW781_09 | 1 | 26.67 (72) | 20 (60) | 10 (25) | 10.8 (82) | 3.9 (46) | 2.23 (44) |
| SW781_09 | 2 | 36.67 (91) | 56.67 (99) | 10 (25) | 13.2 (93) | 21.82 (102) | 1.02 (10) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW781_09 | 3 | 40 (96) | 13.33 (38) | 6.67 (14) | 10.01 (78) | 3.6 (43) | 1.2 (16) |
| SW782_02 | 1 | 6.67 (5) | 30 (78) | 26.67 (76) | 4.04 (26) | 7.92 (76) | 7.83 (97) |
| SW782_02 | 2 | 13.33 (35) | 46.67 (94) | 53.33 (106) | 5.27 (43) | 14.48 (94) | 24.14 (112) |
| SW782_02 | 3 | 13.33 (35) | 33.33 (79) | 40 (96) | 4.05 (27) | 18.84 (98) | 12.27 (105) |
| SW782_03 | 1 | 40 (91) | 60 (99) | 53.33 (102) | 6.08 (50) | 22.19 (98) | 9.23 (99) |
| SW782_03 | 2 | 46.67 (97) | 33.33 (78) | 46.67 (100) | 10.21 (78) | 9.99 (82) | 10.75 (101) |
| SW782_03 | 3 | 56.67 (107) | 76.67 (110) | 20 (56) | 13.51 (91) | 22.69 (100) | 6.06 (88) |
| SW786_02 | 1 | 10 (13) | 16.67 (54) | 13.33 (38) | 2.97 (14) | 7.06 (68) | 4.46 (78) |
| SW786_02 | 2 | 10 (13) | 13.33 (37) | 6.67 (13) | 2.71 (9) | 8.22 (74) | 3.61 (68) |
| SW786_02 | 3 | 13.33 (34) | 10 (22) | 3.33 (5) | 4.9 (39) | 4.08 (49) | 2.19 (42) |
| SW788_05 | 1 | 0 (1) | 6.67 (9) | 3.33 (5) | 2.64 (7) | 1.43 (2) | 1.04 (12) |
| SW788_05 | 2 | 6.67 (4) | 0 (1) | 3.33 (5) | 4.05 (23) | 1.6 (5) | 1 (8) |
| SW788_05 | 3 | 13.33 (31) | 6.67 (9) | 6.67 (12) | 4.07 (25) | 2.43 (25) | 0.69 (3) |
| SW790_01 | 1 | 6.67 (4) | 20 (53) | 23.33 (62) | 2.22 (4) | 3.49 (38) | 3.35 (61) |
| SW790_01 | 2 | 16.67 (43) | 13.33 (34) | 20 (50) | 5.36 (35) | 2.43 (24) | 4.35 (73) |
| SW790_01 | 3 | 33.33 (74) | 10 (19) | 33.33 (81) | 14.55 (84) | 1.67 (6) | 4.76 (72) |
| SW790_03 | 1 | 10 (10) | 16.67 (48) | 6.67 (11) | 7.04 (48) | 6.49 (59) | 4.27 (70) |
| SW790_03 | 2 | 26.67 (60) | 20 (51) | 13.33 (33) | 10.02 (65) | 6.51 (59) | 2.64 (52) |
| SW790_03 | 3 | 43.33 (83) | 26.67 (65) | 16.67 (41) | 13.51 (80) | 11.34 (73) | 3.4 (62) |
| SW790_04 | 1 | 6.67 (4) | 86.67 (97) | 76.67 (94) | 3.59 (18) | 39.95 (92) | 21.81 (92) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW790_04 | 2 | 10 (10) | 96.67 (99) | 63.33 (92) | 7.03 (46) | 61.95 (99) | 22.27 (93) |
| SW790_04 | 3 | 10 (10) | 73.33 (95) | 83.33 (96) | 3.82 (20) | 37.69 (92) | 32.25 (94) |
| SW793_02 | 1 | 10 (9) | 33.33 (66) | 10 (19) | 1.7 (2) | 11.4 (72) | 2.58 (49) |
| SW793_02 | 2 | 10 (9) | 6.67 (8) | 16.67 (39) | 4.02 (18) | 1.96 (8) | 3.91 (65) |
| SW793_02 | 3 | 20 (41) | 3.33 (1) | 6.67 (11) | 14.69 (77) | 3.78 (38) | 2.51 (45) |
| SW793_03 | 1 | 26.67 (54) | 10 (17) | 20 (44) | 14.62 (76) | 2.15 (11) | 2.05 (37) |
| SW793_03 | 2 | 30 (60) | 10 (17) | 26.67 (60) | 12.52 (69) | 4.07 (39) | 3.38 (56) |
| SW793_03 | 3 | 43.33 (76) | 23.33 (56) | 26.67 (61) | 15.05 (77) | 4.87 (48) | 2.55 (45) |
| SW795_01 | 1 | 13.33 (23) | 13.33 (29) | 0 (1) | 4.35 (24) | 5.97 (52) | 0.21 (1) |
| SW795_01 | 2 | 13.33 (23) | 10 (17) | 13.33 (30) | 3.59 (16) | 4.45 (40) | 3.82 (58) |
| SW795_01 | 3 | 13.33 (23) | 13.33 (28) | 30 (66) | 3.18 (14) | 4.97 (45) | 3.54 (55) |
| SW795_04 | 1 | 13.33 (23) | 6.67 (7) | 10 (17) | 8.46 (46) | 2.91 (30) | 1.63 (17) |
| SW795_04 | 2 | 20 (37) | 3.33 (1) | 13.33 (29) | 9.84 (55) | 2.35 (17) | 2.23 (36) |
| SW795_04 | 3 | 23.33 (45) | 6.67 (7) | 20 (41) | 13.26 (68) | 4.84 (42) | 2.48 (41) |
| SW795_08 | 1 | 10 (9) | 10 (14) | 10 (17) | 3.12 (13) | 6.84 (48) | 1.94 (32) |
| SW795_08 | 2 | 10 (9) | 26.67 (51) | 23.33 (48) | 2.94 (9) | 9.26 (56) | 2.55 (38) |
| SW795_08 | 3 | 16.67 (31) | 6.67 (6) | 16.67 (33) | 3.35 (13) | 4.47 (38) | 2.16 (34) |
| SW795_10 | 1 | 10 (9) | 3.33 (1) | 0 (1) | 4.24 (17) | 1.16 (1) | 1.9 (30) |
| SW795_10 | 2 | 10 (9) | 10 (11) | 3.33 (3) | 5.38 (23) | 2.25 (14) | 1.06 (9) |
| SW795_10 | 3 | 16.67 (30) | 6.67 (6) | 10 (17) | 9.6 (50) | 3.04 (29) | 1.74 (24) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW796_03 | 1 | 3.33 (1) | 13.33 (20) | 6.67 (8) | 2.74 (7) | 2.41 (14) | 1.63 (15) |
| SW796_03 | 2 | 10 (9) | 13.33 (20) | 6.67 (8) | 5.43 (24) | 2.46 (19) | 1.43 (13) |
| SW796_03 | 3 | 33.33 (52) | 13.33 (20) | 20 (35) | 12.56 (59) | 1.58 (3) | 1.63 (17) |
| SW796_04 | 1 | 20 (29) | 36.67 (51) | 50 (68) | 3.08 (10) | 14.09 (58) | 8.81 (64) |
| SW796_04 | 2 | 33.33 (49) | 36.67 (51) | 56.67 (68) | 4.66 (17) | 8.58 (47) | 14.1 (67) |
| SW796_04 | 3 | 50 (61) | 53.33 (59) | 80 (72) | 11.29 (51) | 26.02 (65) | 36.52 (73) |
| SW796_05 | 1 | 36.67 (52) | 76.67 (70) | 66.67 (68) | 8.56 (36) | 59.66 (71) | 35.5 (70) |
| SW796_05 | 2 | 50 (60) | 66.67 (66) | 43.33 (63) | 7.04 (30) | 45.77 (70) | 10.83 (66) |
| SW796_05 | 3 | 76.67 (69) | 56.67 (59) | 40 (61) | 49.24 (70) | 26.38 (64) | 14.94 (66) |
| SW796_09 | 1 | 40 (54) | 23.33 (40) | 70 (67) | 19.14 (58) | 14.02 (56) | 24.07 (67) |
| SW796_09 | 2 | 50 (58) | 13.33 (20) | 83.33 (67) | 21.5 (60) | 4.87 (34) | 33.74 (67) |
| SW796_09 | 3 | 50 (58) | 16.67 (29) | 86.67 (67) | 33.42 (61) | 13.44 (54) | 50.88 (67) |
| SW797_06 | 1 | 10 (8) | 26.67 (41) | 33.33 (55) | 1.78 (2) | 9.6 (48) | 6.61 (57) |
| SW797_06 | 2 | 23.33 (35) | 43.33 (52) | 13.33 (23) | 36.92 (63) | 26.56 (61) | 7.52 (59) |
| SW797_06 | 3 | 23.33 (35) | 23.33 (38) | 10 (13) | 16.58 (56) | 18.93 (58) | 7.7 (61) |
| SW797_10 | 1 | 26.67 (36) | 13.33 (20) | 20 (31) | 6.68 (27) | 2.88 (23) | 2.44 (30) |
| SW797_10 | 2 | 30 (41) | 10 (11) | 30 (50) | 9.1 (36) | 2.59 (19) | 2.72 (36) |
| SW797_10 | 3 | 30 (42) | 20 (31) | 26.67 (44) | 34.65 (59) | 3.7 (27) | 3.25 (39) |
| SW798_06 | 1 | 6.67 (3) | 23.33 (35) | 10 (13) | 3.01 (8) | 7.27 (37) | 2.25 (28) |
| SW798_06 | 2 | 20 (27) | 33.33 (37) | 13.33 (21) | 8.41 (31) | 5.63 (35) | 0.88 (3) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW798_06 | 3 | 20 (27) | 13.33 (19) | 10 (13) | 10.11 (38) | 3.94 (26) | 1.64 (14) |
| SW802_01 | 1 | 13.33 (15) | 56.67 (46) | 43.33 (52) | 4.17 (11) | 12.35 (44) | 1.96 (23) |
| SW802_01 | 2 | 30 (38) | 40 (41) | 26.67 (40) | 5.94 (22) | 7.39 (37) | 0.89 (3) |
| SW802_01 | 3 | 36.67 (41) | 60 (48) | 20 (28) | 5.47 (18) | 7.78 (37) | 0.79 (2) |
| SW802_06 | 1 | 13.33 (15) | 13.33 (18) | 10 (13) | 8.43 (28) | 7.88 (36) | 1.03 (5) |
| SW802_06 | 2 | 13.33 (15) | 6.67 (5) | 23.33 (33) | 10.18 (34) | 2.17 (10) | 1.66 (12) |
| SW802_06 | 3 | 20 (26) | 10 (11) | 20 (28) | 15.35 (46) | 3.73 (25) | 1.87 (20) |
| SW803_02 | 1 | 40 (39) | 13.33 (16) | 6.67 (7) | 11.27 (35) | 2.23 (11) | 1.86 (16) |
| SW803_02 | 2 | 50 (43) | 10 (10) | 3.33 (3) | 7.87 (27) | 3.18 (22) | 1.19 (5) |
| SW803_02 | 3 | 53.33 (43) | 3.33 (1) | 16.67 (20) | 9.3 (30) | 2.32 (11) | 1.34 (6) |
| SW803_03 | 1 | 10 (7) | 16.67 (20) | 0 (1) | 4.67 (14) | 2.42 (11) | 0.28 (1) |
| SW803_03 | 2 | 10 (7) | 20 (21) | 3.33 (2) | 4.42 (13) | 2.66 (16) | 1.47 (6) |
| SW803_03 | 3 | 20 (22) | 50 (36) | 10 (9) | 11.47 (32) | 2.53 (14) | 2.27 (15) |
| SW803_07 | 1 | 16.67 (19) | 3.33 (1) | 25 (28) | 5.25 (14) | 2.45 (13) | 3.85 (26) |
| SW803_07 | 2 | 20 (21) | 6.67 (3) | 16.67 (16) | 4.83 (13) | 5.28 (22) | 3.3 (23) |
| SW803_07 | 3 | 33.33 (32) | 10 (9) | 3.33 (2) | 9.52 (28) | 3.13 (16) | 1.84 (12) |
| SW803_09 | 1 | 10 (7) | 10 (6) | 6.67 (3) | 2.73 (5) | 1.99 (6) | 1.64 (6) |
| SW803_09 | 2 | 13.33 (13) | 6.67 (3) | 3.33 (2) | 7.69 (22) | 1.49 (1) | 0.97 (1) |
| SW803_09 | 3 | 46.67 (32) | 10 (6) | 6.67 (3) | 25.95 (36) | 1.75 (3) | 0.99 (1) |
| SW805_01 | 1 | 13.33 (12) | 33.33 (20) | 13.33 (10) | 6.29 (17) | 8.93 (21) | 8.09 (35) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW805_01 | 2 | 23.33 (20) | 13.33 (8) | 16.67 (13) | 8.66 (21) | 5.53 (19) | 7.59 (34) |
| SW805_01 | 3 | 46.67 (32) | 6.67 (3) | 26.67 (24) | 36.51 (36) | 6.07 (20) | 20.44 (39) |
| SW805_03 | 1 | 3.33 (1) | 6.67 (3) | 20 (13) | 2.33 (2) | 2.42 (7) | 1.31 (2) |
| SW805_03 | 2 | 6.67 (3) | 6.67 (3) | 0 (1) | 2.56 (3) | 2.15 (6) | 1.52 (4) |
| SW805_03 | 3 | 13.33 (10) | 3.33 (1) | 26.67 (20) | 6.28 (14) | 2.2 (6) | 1.38 (2) |
| SW805_05 | 1 | 10 (5) | 13.33 (5) | 33.33 (26) | 6.85 (14) | 3.39 (9) | 6.1 (28) |
| SW805_05 | 2 | 20 (14) | 13.33 (5) | 3.33 (1) | 11.81 (22) | 4.29 (10) | 5.07 (21) |
| SW805_05 | 3 | 33.33 (21) | 3.33 (1) | 20 (12) | 18.11 (26) | 4.99 (12) | 5.95 (26) |
| SW805_10 | 1 | 10 (5) | 10 (1) | 26.67 (18) | 4.07 (7) | 1.51 (1) | 3.26 (15) |
| SW805_10 | 2 | 10 (5) | 13.33 (3) | 20 (12) | 5.95 (12) | 1.98 (3) | 2.62 (11) |
| SW805_10 | 3 | 13.33 (7) | 10 (1) | 23.33 (15) | 4.26 (8) | 1.84 (2) | 2.57 (9) |
| SW806_08 | 1 | 3.33 (1) | 33.33 (10) | 10 (3) | 3.78 (4) | 14.75 (16) | 1.74 (2) |
| SW806_08 | 2 | 6.67 (2) | 53.33 (17) | 23.33 (14) | 1.37 (1) | 22.87 (21) | 8.82 (25) |
| SW806_08 | 3 | 26.67 (12) | 20 (4) | 10 (3) | 7.49 (12) | 9.04 (12) | 1.03 (1) |
| SW809_02 | 1 | 13.33 (5) | 23.33 (8) | 6.67 (1) | 12.53 (16) | 12.61 (15) | 1.81 (3) |
| SW809_02 | 2 | 26.67 (9) | 16.67 (3) | 16.67 (7) | 11.76 (15) | 4.77 (8) | 3.64 (11) |
| SW809_02 | 3 | 30 (12) | 36.67 (10) | 26.67 (11) | 13.9 (16) | 11.8 (13) | 6.22 (19) |
| SW809_04 | 1 | 50 (15) | 63.33 (15) | 20 (8) | 13.97 (16) | 7.39 (10) | 1.73 (1) |
| SW809_04 | 2 | 73.33 (18) | 33.33 (8) | 33.33 (14) | 20.3 (17) | 2.59 (4) | 2.61 (5) |
| SW809_04 | 3 | 80 (19) | 40 (9) | 43.33 (16) | 38.74 (18) | 3.96 (5) | 4.34 (11) |

| Genotypes | Plate | Vision evaluation | | | Image analysis | | |
|---|---|---|---|---|---|---|---|
| | | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) | Exp 1 (rank) | Exp 2 (rank) | Exp 3 (rank) |
| SW809_06 | 1 | 6.67 (1) | 63.33 (13) | 10 (2) | 2.98 (3) | 19.51 (13) | 1.79 (1) |
| SW809_06 | 2 | 23.33 (7) | 33.33 (8) | 26.67 (9) | 5.38 (5) | 17.34 (11) | 7.12 (15) |
| SW809_06 | 3 | 33.33 (10) | 56.67 (10) | 20 (7) | 12.9 (13) | 11.73 (9) | 5.39 (12) |
| SW809_08 | 1 | 10 (2) | 13.33 (2) | 6.67 (1) | 4.06 (3) | 2.01 (2) | 2.69 (5) |
| SW809_08 | 2 | 16.67 (4) | 20 (2) | 13.33 (2) | 5.74 (4) | 5.25 (4) | 2.67 (3) |
| SW809_08 | 3 | 36.67 (9) | 20 (2) | 10 (1) | 11.06 (11) | 4.71 (4) | 1.88 (1) |
| SWG39_01 | 1 | 63.33 (9) | 66.67 (8) | 43.33 (10) | 8.84 (6) | 34.4 (9) | 5.16 (7) |
| SWG39_01 | 2 | 63.33 (9) | 70 (9) | 46.67 (10) | 5.84 (4) | 44.22 (10) | 5.74 (8) |
| SWG39_01 | 3 | 73.33 (11) | 76.67 (11) | 60 (11) | 11.04 (8) | 48.71 (11) | 5.33 (7) |
| SWG39_05 | 1 | 6.67 (1) | 20 (2) | 40 (8) | 2.67 (1) | 2.44 (2) | 3.28 (3) |
| SWG39_05 | 2 | 13.33 (1) | 10 (1) | 13.33 (1) | 4.23 (1) | 1.65 (1) | 2.47 (1) |
| SWG39_05 | 3 | 10 (2) | 20 (2) | 10 (1) | 2.79 (2) | 2.81 (3) | 2.03 (1) |
| WS98.SB_01 | 1 | 16.67 (1) | 66.67 (4) | 16.67 (1) | 7.35 (1) | 43.13 (4) | 3.79 (1) |
| WS98.SB_01 | 2 | 26.67 (2) | 70 (6) | 36.67 (6) | 9.28 (2) | 32.5 (5) | 4.22 (3) |
| WS98.SB_01 | 3 | 26.67 (2) | 56.67 (4) | 30 (4) | 9.79 (2) | 18.81 (4) | 2.99 (1) |
| WS98.SB_07 | 1 | 26.67 (1) | 23.33 (1) | 26.67 (1) | 19.77 (1) | 7.32 (1) | 10.03 (1) |
| WS98.SB_07 | 2 | 43.33 (2) | 46.67 (3) | 30 (3) | 37 (2) | 15.38 (3) | 7.68 (2) |
| WS98.SB_07 | 3 | 70 (2) | 40 (2) | 20 (1) | 46.9 (2) | 10.33 (2) | 5.45 (1) |

**Java code for ImageJ analysis for detached leaf and leaf disk assay**

**For each leaf**

```
dir= getDirectory("");
// Get files in directory:
	files= getFileList(dir);
	path = dir + files[k];
	open(path);
	run("Rotate 90 Degrees Right");
//decode QR
	makeRectangle(414, 216, 990, 996);
	run("Duplicate...", " ");
	run("8-bit");
	//run("Brightness/Contrast...");
	setMinAndMax(204, 206);
	run("Apply LUT");
	run("QR Decoder", "error=FAILED");
	selectWindow("QR Code");
	QR_ID = getInfo("window.contents");
	run("Close");
	print(QR_ID);
	run("Close");
//analyze lesions on the leaf
	selectWindow(files[k]);
	makeRectangle(1476, 336, 5010, 1032);
	run("Duplicate...", " ");
	run("Duplicate...", " ");
	run("8-bit");
	setAutoThreshold("Default");
	//run("Threshold...");
	setThreshold(0, 188);
	//setThreshold(0, 188);
	setOption("BlackBackground", false);
	run("Convert to Mask");
	run("Analyze Particles...", "size=0-Infinity display include summarize");
	close();
	selectWindow("Summary");
	lines = split(getInfo(), "\n");
	headings = split(lines[0], "\t");
	values = split(lines[lengthOf(lines)-1], "\t");
	 for (i=0; i<headings.length; i++)
	     print(headings[i]+": "+values[i]);
	// Color Thresholder 1.51j
	// Autogenerated macro, single images only!
```

```
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=0;
max[0]=35;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=0;
max[2]=210;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------

run("Analyze Particles...", "display include summarize");
close();
```

```
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
```

**Code for leaf disk assay**

```
//extract each disk from 28 disk in each plate
        //run("Subtract Background...", "rolling=60");
        // Color Thresholder 1.51j
        // Autogenerated macro, single images only!
        min=newArray(3);
        max=newArray(3);
        filter=newArray(3);
        a=getTitle();
        run("HSB Stack");

        run("Convert Stack to Images");
        selectWindow("Hue");
        rename("0");
        selectWindow("Saturation");
        rename("1");
        selectWindow("Brightness");
        rename("2");
        min[0]=0;
        max[0]=126;
        filter[0]="pass";
        min[1]=150;
        max[1]=255;
        filter[1]="pass";
        min[2]=56;
        max[2]=255;
        filter[2]="pass";
        filter[0]="pass";
        filter[1]="pass";
        filter[2]="pass";
        for (i=0;i<3;i++){
          selectWindow(""+i);
          setThreshold(min[i], max[i]);
          run("Convert to Mask");
          if (filter[i]=="stop")  run("Invert");
        }
        imageCalculator("AND create", "0","1");
```

```
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=1000-Infinity circularity=0.00-1.00 include
clear include add");
//try to rename
//for ( i =0; i < roiManager("count"); i++) {
    //roiManager ("select", i);
    //yyyy=  split( call( "ij.plugin.frame.RoiManager.getName", i ), "-" );
    //roiManager( "Rename",yyyy[1] );
//}
//roiManager("Sort");
close();
for (i=0; i<roiManager("count"); ++i) {
//Select the original which should be cropped
   run("Duplicate...", "title=crop");
   roiManager("Select", i);
   run("Crop");
   saveAs("[file_name]".tif");
 }
//analyze each disk
////////////////////////////////////////////////total area
showProgress(k, files.length);
      IJ.redirectErrorMessages();
      open(path);
      if (nImages>=1) {
//remove background
setBackgroundColor(0, 0, 0);
run("Clear Outside");


// Color Thresholder 1.51j
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
```

```
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=0;
max[0]=255;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=1;
max[2]=255;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=3000-Infinity include summarize");
close();
print("total_area");
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
```

```
} else
        print("Error opening "+path);
////////////////////////////////////////////yellow area
showProgress(k, files.length);
      IJ.redirectErrorMessages();
      open(path);
      if (nImages>=1) {
//remove background
setBackgroundColor(0, 0, 0);
run("Clear Outside");
// Color Thresholder 1.51j
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=0;
max[0]=43;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=164;
max[2]=255;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
```

```
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=10-Infinity include summarize");
close();
print("yellow");
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
} else
            print("Error opening "+path);
//////////////////////////////////////////////black area
showProgress(k, files.length);
        IJ.redirectErrorMessages();
        open(path);
        if (nImages>=1) {
//remove background
setBackgroundColor(0, 0, 0);
run("Clear Outside");
// Color Thresholder 1.51j
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=0;
max[0]=33;
filter[0]="pass";
min[1]=0;
```

```
max[1]=255;
filter[1]="pass";
min[2]=1;
max[2]=155;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=20-Infinity include summarize");
close();
print("black");
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
} else
           print("Error opening "+path);
//////////////////////////////////////////////black_dot
showProgress(k, files.length);
        IJ.redirectErrorMessages();
        open(path);
        if (nImages>=1) {

//remove background
setBackgroundColor(0, 0, 0);
run("Clear Outside");
```

```
// Color Thresholder 1.51j
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=0;
max[0]=33;
filter[0]="pass";
min[1]=0;
max[1]=255;
filter[1]="pass";
min[2]=1;
max[2]=155;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
```

```
run("Analyze Particles...", "size=20-1000 circularity=0.30-1.00 show=Nothing
include summarize");
close();
print("black_dot");
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
} else
            print("Error opening "+path);
////////////////////////////////////////////////green area
showProgress(k, files.length);
        IJ.redirectErrorMessages();
        open(path);
        if (nImages>=1) {
//remove background
setBackgroundColor(0, 0, 0);
run("Clear Outside");
// Color Thresholder 1.51j
// Autogenerated macro, single images only!
min=newArray(3);
max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue");
rename("0");
selectWindow("Saturation");
rename("1");
selectWindow("Brightness");
rename("2");
min[0]=37;
max[0]=135;
filter[0]="pass";
min[1]=42;
max[1]=255;
filter[1]="pass";
min[2]=1;
max[2]=255;
filter[2]="pass";
filter[0]="pass";
filter[1]="pass";
```

```
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding----------
run("Analyze Particles...", "size=0-Infinity circularity=0.30-1.00
show=Nothing include summarize");
close();
print("green");
selectWindow("Summary");
lines = split(getInfo(), "\n");
headings = split(lines[0], "\t");
values = split(lines[lengthOf(lines)-1], "\t");
 for (i=0; i<headings.length; i++)
     print(headings[i]+": "+values[i]);
} else
            print("Error opening "+path);
}
```

Table II.2. Phenotype summary of DTI, DTIA, DSVI, DSIA, mean of two locations, NY and PA, and MTL, MNY, and MPA based on each genotype (rank) ordered based on DTIA.

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW43_09 | Upland North | 0.18 (23) | 0.02 (1) | 0.6 (203) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW788_05 | Lowland North | 0.06 (1) | 0.02 (1) | 0 (1) | 0.19 (19) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 3 (409) | 3 (288) |
| SW805_03 | Lowland North | 0.1 (3) | 0.02 (1) | 0 (1) | 0.24 (38) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| SW797_09 | Lowland North | 0.27 (49) | 0.03 (4) | 0.8 (261) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SWG39_05 | Lowland South | 0.15 (14) | 0.03 (4) | 0.2 (89) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| Shelter_07 | Upland East | 0.19 (27) | 0.04 (6) | 0.1 (44) | 0.45 (83) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW803_03 | Lowland North | 0.15 (14) | 0.04 (6) | 0 (1) | 0.26 (46) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW796_03 | Lowland North | 0.14 (6) | 0.04 (6) | 0.3 (119) | 0.53 (93) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 2 (322) | 3 (288) |
| High.Tide_06 | Lowland North | 0.18 (23) | 0.04 (6) | 0.5 (177) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 2 (172) |
| KY1625_05 | Upland East | 0.14 (6) | 0.04 (6) | 0.6 (203) | 0.47 (87) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW799_08 | Lowland North | 0.38 (114) | 0.04 (6) | 0.6 (203) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 3 (288) |
| SW127_06 | Upland West | 0.2 (28) | 0.04 (6) | 1 (394) | 1 (245) | 5 (479) | 5 (396) | 5 (477) | 5 (420) | 4 (457) | 5 (452) |
| KY1625_07 | Upland East | 0.3 (71) | 0.05 (13) | 0.7 (236) | 0.99 (197) | 0 (1) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| SW803_07 | Lowland North | 0.14 (6) | 0.05 (13) | 0.9 (306) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 0 (1) | 0 (1) | 1 (129) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW795_08 | Lowland North | 0.15 (14) | 0.05 (13) | 0.9 (306) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW790_01 | Lowland/Upland Mix | 0.2 (28) | 0.05 (13) | 0.2 (89) | 0.35 (69) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW805_04 | Lowland North | 0.35 (95) | 0.05 (13) | 0.6 (203) | 0.94 (138) | 0 (1) | 1 (22) | 1 (132) | 1 (75) | 0 (1) | 0 (1) |
| Blackwell_04 | Upland West | 0.14 (6) | 0.05 (13) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW65_07 | Upland East | 0.15 (14) | 0.05 (13) | 0.9 (306) | 0.98 (164) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 1 (162) | 3 (288) |
| High.Tide_05 | Lowland North | 0.16 (19) | 0.05 (13) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW786_02 | Upland North | 0.11 (4) | 0.05 (13) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| High.Tide_08 | Lowland North | 0.36 (99) | 0.05 (13) | 0.9 (306) | 0.98 (164) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |
| SW803_02 | Lowland North | 0.21 (34) | 0.05 (13) | 0 (1) | 0.15 (9) | 2 (294) | 5 (396) | 0 (1) | 1 (75) | 5 (473) | 5 (452) |
| SW795_10 | Lowland North | 0.09 (2) | 0.05 (13) | 0 (1) | 0.29 (53) | 2 (294) | 3 (161) | 0 (1) | 1 (75) | 3 (409) | 3 (288) |
| SW798_06 | Lowland North | 0.17 (22) | 0.05 (13) | 0 (1) | 0.12 (2) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 3 (409) | 3 (288) |
| SW795_01 | Lowland North | 0.14 (6) | 0.05 (13) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SWG39_02 | Lowland South | 0.27 (49) | 0.05 (13) | 0.1 (44) | 0.33 (65) | 3 (448) | 4 (309) | 3 (407) | 4 (362) | 3 (409) | 3 (288) |
| SW127_07 | Upland West | 0.23 (41) | 0.06 (28) | 0 (1) | 0.13 (3) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW802_01 | Lowland North | 0.35 (95) | 0.06 (28) | 0.2 (89) | 0.98 (164) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW793_02 | Lowland North | 0.14 (6) | 0.06 (28) | 0.1 (44) | 0.57 (98) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW802_06 | Lowland North | 0.14 (6) | 0.06 (28) | 0 (1) | 0.18 (17) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| SW43_05 | Upland North | 0.27 (49) | 0.06 (28) | 0.9 (306) | 0.88 (127) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 0 (1) |
| SW795_04 | Lowland North | 0.13 (5) | 0.06 (28) | 0.4 (137) | 0.57 (98) | 2 (294) | 3 (161) | 0 (1) | 0 (1) | 3 (409) | 3 (288) |
| SW110_10 | Upland West | 0.28 (63) | 0.06 (28) | 0.5 (177) | 0.99 (197) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 4 (406) |
| SW799_05 | Lowland North | 0.32 (83) | 0.06 (28) | 0.8 (261) | 0.99 (197) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 0 (1) | 1 (129) |
| SW116_03 | Upland North | 0.2 (28) | 0.06 (28) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW803_09 | Lowland North | 0.14 (6) | 0.06 (28) | 0.1 (44) | 0.2 (22) | 3 (448) | 5 (396) | 1 (132) | 2 (150) | 4 (457) | 5 (452) |
| SW805_05 | Lowland North | 0.16 (19) | 0.07 (38) | 0 (1) | 0.16 (11) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW63_02 | Upland North | 0.3 (71) | 0.07 (38) | 1 (394) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| Shelter_09 | Upland East | 0.35 (95) | 0.07 (38) | 0.5 (177) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| High.Tide_09 | Lowland North | 0.33 (84) | 0.07 (38) | 0.4 (137) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 2 (322) | 2 (172) |
| SW110_04 | Upland West | 0.24 (45) | 0.07 (38) | 0.5 (177) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW809_05 | Upland East | 0.38 (114) | 0.07 (38) | 0.4 (137) | 0.68 (107) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 3 (288) |
| SW114_02 | Upland North | 0.18 (23) | 0.07 (38) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 4 (362) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.6_03 | Lowland North | 0.21 (34) | 0.07 (38) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 0 (1) | 0 (1) | 3 (409) | 4 (406) |
| SW805_09 | Lowland North | 0.3 (71) | 0.07 (38) | 0.7 (236) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 1 (75) | 2 (322) | 4 (406) |
| SW806_08 | Lowland North | 0.2 (28) | 0.07 (38) | 0 (1) | 0.2 (22) | 2 (294) | 5 (396) | 2 (286) | 3 (266) | 3 (409) | 5 (452) |
| Blackwell_06 | Upland West | 0.29 (67) | 0.07 (38) | 0.7 (236) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW805_06 | Lowland North | 0.27 (49) | 0.08 (49) | 0.1 (44) | 0.31 (60) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW38_05 | Upland West | 0.2 (28) | 0.08 (49) | 0.9 (306) | 0.98 (164) | 0 (1) | 1 (22) | 0 (1) | 0 (1) | 0 (1) | 1 (129) |
| SW797_10 | Lowland North | 0.23 (41) | 0.08 (49) | 0.1 (44) | 0.27 (49) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| Shelter_08 | Upland East | 0.36 (99) | 0.08 (49) | 0.8 (261) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW793_03 | Lowland North | 0.23 (41) | 0.08 (49) | 0.3 (119) | 0.42 (77) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| ECS.6_02 | Lowland North | 0.26 (47) | 0.08 (49) | 0.4 (137) | 0.87 (126) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| Kanlow_05 | Lowland South | 0.31 (82) | 0.08 (49) | 0.4 (137) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW110_01 | Upland West | 0.27 (49) | 0.08 (49) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 1 (75) | 2 (322) | 3 (288) |
| ECS.11_07 | Upland East | 0.42 (133) | 0.08 (49) | 0 (1) | 0.2 (22) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| SW123_08 | Upland East | 0.3 (71) | 0.08 (49) | 0.9 (306) | 0.99 (197) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 1 (162) | 1 (129) |
| SW65_08 | Upland East | 0.26 (47) | 0.08 (49) | 0.9 (306) | 1 (245) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 1 (129) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.12_06 | Upland West | 0.27 (49) | 0.08 (49) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 0 (1) | 0 (1) | 3 (409) | 5 (452) |
| Carthage_02 | Upland West | 0.29 (67) | 0.08 (49) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 2 (150) | 2 (322) | 4 (406) |
| SW49_01 | Upland North | 0.18 (23) | 0.08 (49) | 0.7 (236) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | NA | NA |
| SW790_03 | Lowland/Upland Mix | 0.2 (28) | 0.08 (49) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 2 (322) | 3 (288) |
| SW799_07 | Lowland North | 0.15 (14) | 0.09 (64) | 0 (1) | 0.16 (11) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW63_07 | Upland North | 0.27 (49) | 0.09 (64) | 0.4 (137) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW129_01 | Upland North | 0.57 (221) | 0.09 (64) | 1 (394) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| Shelter_06 | Upland East | 0.39 (117) | 0.09 (64) | 0 (1) | 1 (245) | 1 (101) | 4 (309) | 0 (1) | 1 (75) | 2 (322) | 4 (406) |
| SW781_05 | Lowland North | 0.21 (34) | 0.09 (64) | 0.1 (44) | 0.14 (5) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 2 (322) | 2 (172) |
| SW123_10 | Upland East | 0.33 (84) | 0.09 (64) | 0.6 (203) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW806_04 | Lowland North | 0.4 (118) | 0.09 (64) | 0.1 (44) | 0.34 (68) | 1 (101) | 4 (309) | 4 (458) | 4 (362) | 0 (1) | 0 (1) |
| High.Tide_01 | Lowland North | 0.27 (49) | 0.09 (64) | 0.1 (44) | 0.29 (53) | 2 (294) | 2 (54) | 1 (132) | 2 (150) | 2 (322) | 2 (172) |
| SW33_03 | Upland West | 0.33 (84) | 0.09 (64) | 0.4 (137) | 0.59 (102) | 2 (294) | 3 (161) | 1 (132) | 2 (150) | 3 (409) | 3 (288) |
| Carthage_05 | Upland West | 0.16 (19) | 0.09 (64) | 0.9 (306) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 2 (322) | 3 (288) |
| SW43_02 | Upland North | 0.3 (71) | 0.09 (64) | 0.7 (236) | 0.99 (197) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 2 (172) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Carthage_04 | Upland West | 0.33 (84) | 0.09 (64) | 0.9 (306) | 1 (245) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 1 (162) | 2 (172) |
| SW788_07 | Lowland North | 0.27 (49) | 0.1 (76) | 0.1 (44) | 0.22 (33) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| KY1625_02 | Upland East | 0.27 (49) | 0.1 (76) | 0.8 (261) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| ECS.12_05 | Upland West | 0.35 (95) | 0.1 (76) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW781_04 | Lowland North | 0.33 (84) | 0.1 (76) | 0.1 (44) | 0.22 (33) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW781_08 | Lowland North | 0.3 (71) | 0.1 (76) | 0.8 (261) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 1 (75) | 1 (162) | 2 (172) |
| SW798_10 | Lowland North | 0.27 (49) | 0.1 (76) | 0.6 (203) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 1 (129) |
| SW805_08 | Lowland North | 0.27 (49) | 0.1 (76) | 0.1 (44) | 0.26 (46) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| SW116_05 | Upland North | 0.4 (118) | 0.1 (76) | 0.1 (44) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| Shelter_01 | Upland East | 0.25 (46) | 0.1 (76) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| SW789_01 | Lowland/Upland Mix | 0.3 (71) | 0.11 (85) | 0.4 (137) | 0.6 (103) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| High.Tide_04 | Lowland North | 0.21 (34) | 0.11 (85) | 0.1 (44) | 0.28 (50) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW788_10 | Lowland North | 0.38 (114) | 0.11 (85) | 0.1 (44) | 0.29 (53) | 1 (101) | 2 (54) | 1 (132) | 1 (75) | 1 (162) | 2 (172) |
| SW805_01 | Lowland North | 0.21 (34) | 0.11 (85) | 0.9 (306) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW782_01 | Upland East | 0.43 (143) | 0.11 (85) | 0.1 (44) | 0.2 (22) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW806_02 | Lowland North | 0.22 (39) | 0.11 (85) | 0.1 (44) | 0.36 (70) | 2 (294) | 5 (396) | 1 (132) | 2 (150) | 4 (457) | 5 (452) |
| SW782_02 | Upland East | 0.29 (67) | 0.11 (85) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 2 (150) | 2 (322) | 4 (406) |
| Cave.in.Rock_07 | Upland East | 0.37 (103) | 0.11 (85) | 0.1 (44) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SW782_03 | Upland East | 0.46 (159) | 0.12 (93) | 0.4 (137) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW58_05 | Upland West | 0.67 (293) | 0.12 (93) | 0 (1) | 0.21 (29) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW127_08 | Upland West | 0.3 (71) | 0.12 (93) | 0.8 (261) | 0.95 (139) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW803_10 | Lowland North | 0.58 (238) | 0.12 (93) | 0 (1) | 0.24 (38) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| Dacotah_07 | Upland North | 0.28 (63) | 0.12 (93) | 0.2 (89) | 0.52 (92) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| SW796_01 | Lowland North | 0.48 (172) | 0.12 (93) | 0.4 (137) | 0.62 (104) | 1 (101) | 4 (309) | 1 (132) | 2 (150) | 1 (162) | 4 (406) |
| SW33_04 | Upland West | 0.36 (99) | 0.12 (93) | 0.4 (137) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW122_06 | Upland West | 0.36 (99) | 0.12 (93) | 0.4 (137) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW64_06 | Upland East | 0.23 (41) | 0.12 (93) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| KY1625_10 | Upland East | 0.5 (178) | 0.12 (93) | 0.4 (137) | 1 (245) | 2 (294) | 3 (161) | 0 (1) | 1 (75) | 3 (409) | 3 (288) |
| SWG32_01 | Lowland South | 0.42 (133) | 0.12 (93) | 0 (1) | 0.18 (17) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 1 (129) |
| SW123_06 | Upland East | 0.53 (198) | 0.13 (104) | 0.8 (261) | 0.98 (164) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW803_06 | Lowland North | 0.47 (160) | 0.13 (104) | 0.2 (89) | 1 (245) | 0 (1) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| SW786_04 | Upland North | 0.43 (143) | 0.13 (104) | 0.4 (137) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW796_04 | Lowland North | 0.44 (154) | 0.13 (104) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW798_09 | Lowland North | 0.27 (49) | 0.13 (104) | 0 (1) | 0.14 (5) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| WS4U_08 | Upland North | 0.33 (84) | 0.13 (104) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| ECS.11_02 | Upland East | 0.43 (143) | 0.13 (104) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| KY1625_04 | Upland East | 0.37 (103) | 0.13 (104) | 0.6 (203) | 0.74 (113) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 3 (288) |
| ECS.10_07 | Upland East | 0.33 (84) | 0.13 (104) | 0 (1) | 0.17 (15) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 2 (172) |
| SW795_02 | Lowland North | 0.45 (156) | 0.14 (113) | 0.2 (89) | 0.45 (83) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| WS98.SB_02 | Upland North | 0.37 (103) | 0.14 (113) | 0.1 (44) | 0.19 (19) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW809_02 | Upland East | 0.5 (178) | 0.14 (113) | 0.7 (236) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 1 (162) | 3 (288) |
| Kanlow_09 | Lowland South | 0.42 (133) | 0.14 (113) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW797_06 | Lowland North | 0.22 (39) | 0.14 (113) | 0.6 (203) | 0.95 (139) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW805_02 | Lowland North | 0.42 (133) | 0.14 (113) | 0 (1) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| KY1625_09 | Upland East | 0.47 (160) | 0.14 (113) | 0.9 (306) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 2 (150) | 3 (409) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS98.SB_01 | Upland Mix | 0.37 (103) | 0.14 (113) | 0.9 (306) | 0.97 (155) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SW795_09 | Lowland North | 0.45 (156) | 0.14 (113) | 0.3 (119) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW788_01 | Lowland North | 0.48 (172) | 0.15 (122) | 0.1 (44) | 0.29 (53) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SWG32_02 | Lowland South | 0.65 (283) | 0.15 (122) | 0.1 (44) | 0.32 (62) | 1 (101) | 4 (309) | 1 (132) | 1 (75) | 1 (162) | 4 (406) |
| SW50_05 | Upland West | 0.37 (103) | 0.15 (122) | 0.9 (306) | 0.98 (164) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| ECS.10_03 | Upland East | 0.4 (118) | 0.15 (122) | 0.1 (44) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 4 (362) | 1 (162) | 2 (172) |
| Cave.in.Rock_04 | Upland East | 0.44 (154) | 0.15 (122) | 0.9 (306) | 0.99 (197) | 2 (294) | 5 (396) | 0 (1) | 1 (75) | 4 (457) | 5 (452) |
| Cave.in.Rock_05 | Upland East | 0.29 (67) | 0.16 (127) | 0.6 (203) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| ECS.11_04 | Upland East | 0.4 (118) | 0.16 (127) | 0.8 (261) | 0.82 (120) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW798_01 | Lowland North | 0.45 (156) | 0.16 (127) | 0.3 (119) | 1 (245) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 1 (129) |
| SW46_06 | Upland North | 0.47 (160) | 0.16 (127) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 1 (162) | 2 (172) |
| SW803_08 | Lowland North | 0.28 (63) | 0.16 (127) | 0 (1) | 0.2 (22) | 2 (294) | 5 (396) | 0 (1) | 0 (1) | 4 (457) | 5 (452) |
| SW109_07 | Upland West | 0.4 (118) | 0.16 (127) | 0.5 (177) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 4 (362) | 3 (409) | 4 (406) |
| SW799_03 | Lowland North | 0.33 (84) | 0.17 (133) | 0.7 (236) | 0.99 (197) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| High.Tide_10 | Lowland North | 0.53 (198) | 0.17 (133) | 0.8 (261) | 0.84 (121) | 1 (101) | 3 (161) | 1 (132) | 1 (75) | 1 (162) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW781_07 | Lowland North | 0.43 (143) | 0.17 (133) | 0.3 (119) | 0.98 (164) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| Timber_06 | Lowland South | 0.3 (71) | 0.17 (133) | 0.9 (306) | 0.98 (164) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| High.Tide_03 | Lowland North | 0.28 (63) | 0.17 (133) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| SW788_03 | Lowland North | 0.5 (178) | 0.17 (133) | 0 (1) | 0.22 (33) | 2 (294) | 4 (309) | 1 (132) | 3 (266) | 3 (409) | 4 (406) |
| SWG32_05 | Lowland South | 0.37 (103) | 0.17 (133) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 2 (150) | 2 (322) | 3 (288) |
| SW124_08 | Upland North | 0.5 (178) | 0.17 (133) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SW802_04 | Lowland North | 0.48 (172) | 0.18 (141) | 0.1 (44) | 0.3 (58) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW114_01 | Upland North | 0.68 (318) | 0.18 (141) | 0.5 (177) | 0.98 (164) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| Pathfinder_03 | Upland West | 0.5 (178) | 0.18 (141) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 3 (409) | 3 (288) |
| SW109_03 | Upland North | 0.55 (216) | 0.18 (141) | 0.6 (203) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 0 (1) | 0 (1) |
| SW122_03 | Upland West | 0.47 (160) | 0.18 (141) | 0.1 (44) | 0.46 (86) | 3 (448) | 4 (309) | 2 (286) | 4 (362) | 3 (409) | 4 (406) |
| Shelter_10 | Upland East | 0.33 (84) | 0.19 (146) | 0.4 (137) | 0.56 (95) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| Cave.in.Rock_09 | Upland East | 0.67 (293) | 0.19 (146) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |
| Sunburst_05 | Upland West | 0.43 (143) | 0.19 (146) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 1 (132) | 2 (150) | 4 (457) | 5 (452) |
| Kanlow_10 | Lowland South | 0.42 (133) | 0.19 (146) | 0.6 (203) | 0.98 (164) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 4 (406) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW43_10 | Upland North | 0.47 (160) | 0.19 (146) | 0.4 (137) | 0.7 (110) | 2 (294) | 4 (309) | 4 (458) | 4 (362) | 1 (162) | 2 (172) |
| SW803_05 | Lowland North | 0.53 (198) | 0.2 (151) | 0 (1) | 0.14 (5) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| Shelter_02 | Upland East | 0.48 (172) | 0.2 (151) | 0.6 (203) | 0.99 (197) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW806_05 | Lowland North | 0.4 (118) | 0.2 (151) | 0 (1) | 0.17 (15) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| ECS.1_03 | Lowland North | 0.4 (118) | 0.2 (151) | 0.2 (89) | 0.42 (77) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| Blackwell_05 | Upland West | 0.68 (318) | 0.2 (151) | 0 (1) | 0.2 (22) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 3 (409) | 3 (288) |
| SW65_06 | Upland East | 0.53 (198) | 0.2 (151) | 0.2 (89) | 0.22 (33) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 3 (288) |
| SW129_07 | Upland North | 0.3 (71) | 0.2 (151) | 0.4 (137) | 0.45 (83) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| ECS.2_04 | Upland West | 0.53 (198) | 0.2 (151) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 2 (322) | 3 (288) |
| SW806_03 | Lowland North | 0.42 (133) | 0.2 (151) | 0.4 (137) | 1 (245) | 4 (474) | 5 (396) | 2 (286) | 2 (150) | 5 (473) | 5 (452) |
| WS98.SB_04 | Upland North | 0.42 (133) | 0.21 (160) | 0.3 (119) | 0.96 (144) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW809_08 | Upland East | 0.43 (143) | 0.21 (160) | 0.4 (137) | 1 (245) | 0 (1) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 2 (172) |
| WS98.SB_07 | Upland Mix | 0.4 (118) | 0.21 (160) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| ECS.1_02 | Lowland North | 0.57 (221) | 0.21 (160) | 0.4 (137) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 2 (172) |
| SW806_06 | Lowland North | 0.4 (118) | 0.21 (160) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW43_08 | Upland North | 0.55 (216) | 0.21 (160) | 0.8 (261) | 1 (245) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 2 (172) |
| Cave.in.Rock_06 | Upland East | 0.42 (133) | 0.22 (166) | 0.7 (236) | 0.99 (197) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW799_09 | Lowland North | 0.57 (221) | 0.22 (166) | 0.9 (306) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW31_03 | Upland North | 0.6 (246) | 0.22 (166) | 0.5 (177) | 1 (245) | 0 (1) | 1 (22) | 1 (132) | 1 (75) | 0 (1) | 0 (1) |
| SW793_09 | Lowland North | 0.75 (368) | 0.22 (166) | 0 (1) | 0.16 (11) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| Dacotah_04 | Upland North | 0.37 (103) | 0.22 (166) | 0.3 (119) | 0.92 (135) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW65_05 | Upland East | 0.53 (198) | 0.23 (171) | 0.5 (177) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW51_10 | Upland West | 0.68 (318) | 0.23 (171) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| WS4U_03 | Upland North | 0.47 (160) | 0.23 (171) | 1 (394) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW803_04 | Lowland North | 0.3 (71) | 0.23 (171) | 0.5 (177) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW40_05 | Upland North | 0.55 (216) | 0.23 (171) | 0.9 (306) | 0.96 (144) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW128_03 | Upland West | 0.4 (118) | 0.23 (171) | 0.6 (203) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 1 (75) | 1 (162) | 2 (172) |
| SW787_02 | Upland North | 0.4 (118) | 0.23 (171) | 0.9 (306) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SWG32_03 | Lowland South | 0.37 (103) | 0.23 (171) | 0.5 (177) | 0.77 (115) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 2 (172) |
| SW116_02 | Upland North | 0.7 (328) | 0.23 (171) | 0.6 (203) | 0.79 (116) | 2 (294) | 4 (309) | 4 (458) | 4 (362) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| KY1625_03 | Upland East | 0.53 (198) | 0.23 (171) | 0.5 (177) | 0.5 (90) | 3 (448) | 4 (309) | 3 (407) | 4 (362) | 3 (409) | 4 (406) |
| SW116_01 | Upland North | 0.61 (257) | 0.24 (181) | 0.7 (236) | 0.98 (164) | 0 (1) | 1 (22) | 1 (132) | 1 (75) | 0 (1) | 0 (1) |
| SW123_01 | Upland East | 0.68 (318) | 0.24 (181) | 0.6 (203) | 0.99 (197) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| SW46_05 | Upland North | 0.47 (160) | 0.24 (181) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 1 (129) |
| SW49_02 | Upland North | 0.73 (352) | 0.24 (181) | 0.9 (306) | 0.98 (164) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| ECS.12_01 | Upland West | 0.6 (246) | 0.24 (181) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 4 (406) |
| Sunburst_10 | Upland West | 0.57 (221) | 0.25 (186) | 1 (394) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW797_04 | Lowland North | 0.53 (198) | 0.25 (186) | 0.1 (44) | 0.21 (29) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| Blackwell_07 | Upland West | 0.5 (178) | 0.25 (186) | 0 (1) | 0.25 (40) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 1 (129) |
| SW809_09 | Upland East | 0.47 (160) | 0.25 (186) | 0.6 (203) | 0.8 (117) | 1 (101) | 4 (309) | 1 (132) | 3 (266) | 2 (322) | 4 (406) |
| Blackwell_02 | Upland West | 0.63 (265) | 0.25 (186) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 1 (162) | 1 (129) |
| SW40_07 | Upland North | 0.57 (221) | 0.25 (186) | 0.4 (137) | 1 (245) | 1 (101) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| Timber_01 | Lowland South | 0.43 (143) | 0.25 (186) | 0.1 (44) | 0.25 (40) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW787_03 | Upland North | 0.6 (246) | 0.25 (186) | 0 (1) | 0.21 (29) | 3 (448) | 4 (309) | 3 (407) | 4 (362) | 3 (409) | 3 (288) |
| SW58_06 | Upland West | 0.4 (118) | 0.26 (194) | 0.4 (137) | 0.98 (164) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW782_09 | Upland East | 0.57 (221) | 0.26 (194) | 0.9 (306) | 0.99 (197) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW796_08 | Lowland North | 0.53 (198) | 0.26 (194) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| Shelter_04 | Upland East | 0.42 (133) | 0.26 (194) | 1 (394) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW806_09 | Lowland North | 0.7 (328) | 0.26 (194) | 1 (394) | 1 (245) | 0 (1) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| Timber_02 | Lowland South | 0.57 (221) | 0.26 (194) | 0.6 (203) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW789_08 | Lowland/Upland Mix | 0.53 (198) | 0.26 (194) | 0.1 (44) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 1 (75) | 2 (322) | 3 (288) |
| SW109_09 | Upland West | 0.67 (293) | 0.26 (194) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW40_03 | Upland North | 0.67 (293) | 0.26 (194) | 0.9 (306) | 0.97 (155) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 0 (1) |
| SW43_06 | Upland North | 0.62 (259) | 0.26 (194) | 0 (1) | 0.25 (40) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SW64_09 | Upland East | 0.57 (221) | 0.26 (194) | 0.9 (306) | 0.99 (197) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 2 (172) |
| Timber_07 | Lowland South | 0.5 (178) | 0.26 (194) | 0.2 (89) | 1 (245) | 2 (294) | 2 (54) | 2 (286) | 2 (150) | 1 (162) | 1 (129) |
| SW787_01 | Upland North | 0.43 (143) | 0.26 (194) | 0.1 (44) | 0.37 (71) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 0 (1) | 0 (1) |
| KY1625_08 | Upland East | 0.62 (259) | 0.26 (194) | 0 (1) | 0.39 (72) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 3 (288) |
| SW796_06 | Lowland North | 0.53 (198) | 0.27 (208) | 1 (394) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW795_07 | Lowland North | 0.7 (328) | 0.27 (208) | 1 (394) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Timber_05 | Lowland South | 0.53 (198) | 0.27 (208) | 0 (1) | 0.15 (9) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW797_01 | Lowland North | 0.52 (196) | 0.27 (208) | 0.1 (44) | 0.53 (93) | 2 (294) | 4 (309) | 0 (1) | 0 (1) | 3 (409) | 4 (406) |
| SW112_07 | Upland West | 0.67 (293) | 0.27 (208) | 0.1 (44) | 0.28 (50) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 0 (1) |
| ECS.11_05 | Upland East | 0.5 (178) | 0.27 (208) | 0.9 (306) | 0.98 (164) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 2 (322) | 4 (406) |
| SWG39_01 | Lowland South | 0.66 (292) | 0.27 (208) | 0.8 (261) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 2 (150) | 2 (322) | 3 (288) |
| SW124_01 | Upland North | 0.67 (293) | 0.27 (208) | 0.1 (44) | 0.28 (50) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 1 (129) |
| SW31_02 | Upland North | 0.63 (265) | 0.27 (208) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 2 (172) |
| SW806_07 | Lowland North | 0.4 (118) | 0.27 (208) | 0 (1) | 0.21 (29) | 3 (448) | 5 (396) | 1 (132) | 2 (150) | 5 (473) | 5 (452) |
| SW789_04 | Lowland/Upland Mix | 0.62 (259) | 0.27 (208) | 0.3 (119) | 1 (245) | 3 (448) | 4 (309) | 4 (458) | 4 (362) | 2 (322) | 2 (172) |
| SW63_06 | Upland North | 0.33 (84) | 0.28 (219) | 0.8 (261) | 0.8 (117) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| Sunburst_07 | Upland West | 0.63 (265) | 0.28 (219) | 0.4 (137) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 3 (288) |
| SW127_09 | Upland West | 0.43 (143) | 0.28 (219) | 0.5 (177) | 1 (245) | 2 (294) | 4 (309) | 0 (1) | 1 (75) | 3 (409) | 4 (406) |
| SWG39_09 | Lowland South | 0.7 (328) | 0.28 (219) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 3 (266) | 3 (409) | 4 (406) |
| WS4U_10 | Upland North | 0.6 (246) | 0.28 (219) | 0.7 (236) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 3 (288) |
| SW110_02 | Upland West | 0.37 (103) | 0.28 (219) | 0 (1) | 0.19 (19) | 3 (448) | 4 (309) | 3 (407) | 4 (362) | 3 (409) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.1_05 | Lowland North | 0.43 (143) | 0.29 (225) | 0.2 (89) | 0.42 (77) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW805_07 | Lowland North | 0.5 (178) | 0.29 (225) | 0.2 (89) | 0.33 (65) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 1 (129) |
| SWG39_06 | Lowland South | 0.37 (103) | 0.29 (225) | 0.9 (306) | 0.99 (197) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW802_02 | Lowland North | 0.4 (118) | 0.29 (225) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 1 (75) | 2 (322) | 2 (172) |
| SW114_07 | Upland West | 0.75 (368) | 0.29 (225) | 0 (1) | 0.98 (164) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| SW790_04 | Lowland/Upland Mix | 0.6 (246) | 0.29 (225) | 0.8 (261) | 0.99 (197) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 1 (129) |
| KY1625_01 | Upland East | 0.73 (352) | 0.29 (225) | 0.1 (44) | 0.25 (40) | 2 (294) | 4 (309) | 1 (132) | 2 (150) | 2 (322) | 4 (406) |
| SW112_01 | Upland West | 0.48 (172) | 0.29 (225) | 0.5 (177) | 1 (245) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 2 (172) |
| ECS.1_07 | Lowland North | 0.53 (198) | 0.29 (225) | 0 (1) | 0.25 (40) | 3 (448) | 5 (396) | 0 (1) | 0 (1) | 5 (473) | 5 (452) |
| SW128_05 | Upland West | 0.57 (221) | 0.3 (234) | 0.8 (261) | 0.96 (144) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW112_06 | Upland West | 0.53 (198) | 0.3 (234) | 0.7 (236) | 0.98 (164) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 1 (162) | 3 (288) |
| SW46_10 | Upland North | 0.4 (118) | 0.3 (234) | 0.2 (89) | 0.41 (75) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| ECS.1_06 | Lowland North | 0.33 (84) | 0.3 (234) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 4 (362) | 1 (162) | 4 (406) |
| SW112_05 | Upland West | 0.67 (293) | 0.3 (234) | 0.8 (261) | 1 (245) | 2 (294) | 4 (309) | 0 (1) | 1 (75) | 3 (409) | 4 (406) |
| SW809_04 | Upland East | 0.5 (178) | 0.3 (234) | 0.9 (306) | 0.98 (164) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 2 (322) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW788_04 | Lowland North | 0.63 (265) | 0.31 (240) | 0.6 (203) | 0.92 (135) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW123_09 | Upland East | 0.63 (265) | 0.31 (240) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW793_10 | Lowland North | 0.58 (238) | 0.31 (240) | 0.9 (306) | 0.96 (144) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| SW809_06 | Upland East | 0.63 (265) | 0.31 (240) | 0.5 (177) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 4 (362) | 2 (322) | 4 (406) |
| ECS.12_04 | Upland West | 0.63 (265) | 0.31 (240) | 0.3 (119) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| Pathfinder_07 | Upland West | 0.54 (215) | 0.31 (240) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 0 (1) | 0 (1) | 3 (409) | 4 (406) |
| Kanlow_03 | Lowland South | 0.6 (246) | 0.31 (240) | 0.4 (137) | 0.58 (101) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 2 (322) | 3 (288) |
| Shelter_05 | Upland East | 0.57 (221) | 0.31 (240) | 0.8 (261) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| SW787_09 | Upland North | 0.78 (403) | 0.31 (240) | 0.2 (89) | 1 (245) | 3 (448) | 5 (396) | 5 (477) | 5 (420) | 0 (1) | 1 (129) |
| SW788_02 | Lowland North | 0.52 (196) | 0.32 (249) | 0.4 (137) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW788_08 | Lowland North | 0.62 (259) | 0.32 (249) | 0 (1) | 0.26 (46) | 0 (1) | 1 (22) | 1 (132) | 1 (75) | 0 (1) | 0 (1) |
| ECS.12_03 | Upland West | 0.42 (133) | 0.32 (249) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW58_01 | Upland West | 0.77 (382) | 0.32 (249) | 1 (394) | 1 (245) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 3 (288) |
| SW796_07 | Lowland North | 0.72 (349) | 0.32 (249) | 0.6 (203) | 0.99 (197) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 1 (162) | 4 (406) |
| SW109_01 | Upland West | 0.5 (178) | 0.32 (249) | 1 (394) | 1 (245) | 3 (448) | 5 (396) | 1 (132) | 2 (150) | 4 (457) | 5 (452) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW114_05 | Upland West | 0.7 (328) | 0.32 (249) | 0.4 (137) | 1 (245) | 3 (448) | 5 (396) | 2 (286) | 4 (362) | 4 (457) | 5 (452) |
| Kanlow_01 | Lowland South | 0.53 (198) | 0.33 (256) | 0.3 (119) | 0.41 (75) | 1 (101) | 4 (309) | 0 (1) | 1 (75) | 2 (322) | 4 (406) |
| Carthage_01 | Upland West | 0.57 (221) | 0.33 (256) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 3 (409) | 3 (288) |
| SW64_03 | Upland East | 0.48 (172) | 0.33 (256) | 0.8 (261) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 1 (129) |
| SW124_03 | Upland North | 0.73 (352) | 0.33 (256) | 0.4 (137) | 0.47 (87) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW789_07 | Lowland/Upland Mix | 0.53 (198) | 0.33 (256) | 0.1 (44) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 2 (150) | 3 (409) | 3 (288) |
| Cave.in.Rock_01 | Upland East | 0.62 (259) | 0.33 (256) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 2 (172) |
| SW40_08 | Upland North | 0.27 (49) | 0.33 (256) | 0.8 (261) | 0.99 (197) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 2 (172) |
| SW796_05 | Lowland North | 0.61 (257) | 0.34 (263) | 0.2 (89) | 0.33 (65) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW795_06 | Lowland North | 0.75 (368) | 0.34 (263) | 0.2 (89) | 0.43 (80) | 0 (1) | 1 (22) | 0 (1) | 0 (1) | 0 (1) | 1 (129) |
| SW128_10 | Upland West | 0.5 (178) | 0.34 (263) | 0.2 (89) | 0.98 (164) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW128_08 | Upland West | 0.57 (221) | 0.34 (263) | 0.3 (119) | 0.99 (197) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW789_06 | Lowland/Upland Mix | 0.65 (283) | 0.34 (263) | 0.1 (44) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| Dacotah_08 | Upland North | 0.47 (160) | 0.34 (263) | 0.1 (44) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW114_03 | Upland North | 0.67 (293) | 0.34 (263) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW114_08 | Upland West | 0.47 (160) | 0.34 (263) | 0.8 (261) | 1 (245) | 3 (448) | 5 (396) | 2 (286) | 3 (266) | 4 (457) | 5 (452) |
| SWG32_10 | Lowland South | 0.63 (265) | 0.35 (271) | 0.7 (236) | 0.84 (121) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW65_03 | Upland East | 0.6 (246) | 0.35 (271) | 0.8 (261) | 0.86 (125) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW786_03 | Upland North | 0.73 (352) | 0.35 (271) | 0.8 (261) | 0.98 (164) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 0 (1) |
| KY1625_06 | Upland East | 0.6 (246) | 0.35 (271) | 0.2 (89) | 0.66 (105) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 3 (288) |
| SW112_09 | Upland West | 0.67 (293) | 0.35 (271) | 0.8 (261) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 3 (288) |
| SW122_05 | Upland West | 0.77 (382) | 0.35 (271) | 0.3 (119) | 0.56 (95) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 1 (162) | 4 (406) |
| SW102_01 | Upland North | 0.72 (349) | 0.35 (271) | 0.5 (177) | 0.71 (111) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 3 (288) |
| WS4U_09 | Upland North | 0.47 (160) | 0.35 (271) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 4 (406) |
| Blackwell_09 | Upland West | 0.5 (178) | 0.36 (279) | 0 (1) | 1 (245) | 1 (101) | 4 (309) | 0 (1) | 1 (75) | 1 (162) | 4 (406) |
| SW123_04 | Upland East | 0.63 (265) | 0.36 (279) | 0.3 (119) | 0.97 (155) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| SW46_02 | Upland North | 0.67 (293) | 0.36 (279) | 0.9 (306) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW63_03 | Upland North | 0.7 (328) | 0.36 (279) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| SW33_02 | Upland West | 0.7 (328) | 0.36 (279) | 0.1 (44) | 0.32 (62) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| ECS.11_01 | Upland East | 0.63 (265) | 0.36 (279) | 0.8 (261) | 0.98 (164) | 2 (294) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW46_09 | Upland North | 0.72 (349) | 0.36 (279) | 0.7 (236) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 2 (172) |
| SW782_06 | Upland East | 0.37 (103) | 0.37 (286) | 0.4 (137) | 1 (245) | 0 (1) | 1 (22) | 1 (132) | 1 (75) | 0 (1) | 0 (1) |
| SW50_01 | Upland West | 0.68 (318) | 0.37 (286) | 0.8 (261) | 0.99 (197) | 1 (101) | 4 (309) | 0 (1) | 0 (1) | 3 (409) | 4 (406) |
| SW123_05 | Upland East | 0.62 (259) | 0.37 (286) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW809_10 | Upland East | 0.73 (352) | 0.37 (286) | 0.8 (261) | 1 (245) | 1 (101) | 5 (396) | 1 (132) | 5 (420) | 1 (162) | 5 (452) |
| SW102_06 | Upland North | 0.8 (410) | 0.37 (286) | 0.9 (306) | 0.9 (130) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 1 (129) |
| SW787_05 | Upland North | 0.68 (318) | 0.37 (286) | 1 (394) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 2 (322) | 2 (172) |
| SW124_04 | Upland North | 0.67 (293) | 0.38 (292) | 1 (394) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW64_08 | Upland East | 0.58 (238) | 0.38 (292) | 0 (1) | 0.25 (40) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW787_10 | Upland North | 0.63 (265) | 0.38 (292) | 0.9 (306) | 0.97 (155) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| Blackwell_10 | Upland West | 0.73 (352) | 0.38 (292) | 0.2 (89) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 2 (150) | 2 (322) | 4 (406) |
| SW112_08 | Upland West | 0.57 (221) | 0.38 (292) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW102_02 | Upland North | 0.65 (283) | 0.38 (292) | 0.6 (203) | 0.96 (144) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 1 (129) |
| Pathfinder_05 | Upland West | 0.58 (238) | 0.38 (292) | 0.2 (89) | 0.98 (164) | 2 (294) | 2 (54) | 2 (286) | 2 (150) | 2 (322) | 2 (172) |
| SW127_01 | Upland West | 0.6 (246) | 0.38 (292) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 2 (322) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW788_06 | Lowland North | 0.67 (293) | 0.38 (292) | 0.7 (236) | 0.99 (197) | 4 (474) | 5 (396) | 2 (286) | 2 (150) | 5 (473) | 5 (452) |
| SW802_05 | Lowland North | 0.83 (432) | 0.39 (301) | 0.4 (137) | 0.96 (144) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW781_03 | Lowland North | 0.47 (160) | 0.39 (301) | 0.8 (261) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW123_03 | Upland East | 0.63 (265) | 0.39 (301) | 0.8 (261) | 0.99 (197) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW128_06 | Upland West | 0.6 (246) | 0.39 (301) | 0.9 (306) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 2 (322) | 2 (172) |
| SW798_05 | Lowland North | 0.78 (403) | 0.39 (301) | 0.5 (177) | 0.99 (197) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 1 (129) |
| Carthage_03 | Upland West | 0.82 (422) | 0.39 (301) | 0.7 (236) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 1 (75) | 2 (322) | 4 (406) |
| SW129_03 | Upland North | 0.88 (453) | 0.39 (301) | 0.7 (236) | 0.98 (164) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 2 (172) |
| Kanlow_04 | Lowland South | 0.58 (238) | 0.39 (301) | 0.8 (261) | 1 (245) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 1 (162) | 3 (288) |
| SWG32_04 | Lowland South | 0.55 (216) | 0.39 (301) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 2 (172) |
| ECS.10_10 | Upland East | 0.43 (143) | 0.39 (301) | 0.8 (261) | 1 (245) | 3 (448) | 3 (161) | 3 (407) | 3 (266) | 2 (322) | 3 (288) |
| SW50_06 | Upland West | 0.63 (265) | 0.4 (311) | 0.9 (306) | 1 (245) | 0 (1) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| SW46_03 | Upland North | 0.75 (368) | 0.4 (311) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 0 (1) | 0 (1) |
| SW122_02 | Upland West | 0.75 (368) | 0.4 (311) | 1 (394) | 1 (245) | 4 (474) | 5 (396) | 3 (407) | 4 (362) | 4 (457) | 5 (452) |
| SW799_10 | Lowland North | 0.55 (216) | 0.41 (314) | 0.8 (261) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SWG39_07 | Lowland South | 0.7 (328) | 0.41 (314) | 0.9 (306) | 0.99 (197) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW51_01 | Upland West | 0.67 (293) | 0.41 (314) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| SW115_03 | Upland North | 0.78 (403) | 0.41 (314) | 0.2 (89) | 0.97 (155) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW102_05 | Upland North | 0.77 (382) | 0.41 (314) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 3 (288) |
| SW787_07 | Upland North | 0.75 (368) | 0.41 (314) | 0.2 (89) | 1 (245) | 3 (448) | 5 (396) | 3 (407) | 5 (420) | 3 (409) | 3 (288) |
| SW38_08 | Upland West | 0.5 (178) | 0.41 (314) | 1 (394) | 1 (245) | 3 (448) | 5 (396) | 3 (407) | 5 (420) | 3 (409) | 3 (288) |
| SW102_04 | Upland North | 0.77 (382) | 0.42 (321) | 0.9 (306) | 0.85 (123) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW806_01 | Lowland North | 0.7 (328) | 0.42 (321) | 0.1 (44) | 1 (245) | 0 (1) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW128_07 | Upland West | 0.75 (368) | 0.42 (321) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| Pathfinder_06 | Upland West | 0.68 (318) | 0.42 (321) | 0.3 (119) | 1 (245) | 2 (294) | 4 (309) | 0 (1) | 0 (1) | 4 (457) | 4 (406) |
| SW58_04 | Upland West | 0.5 (178) | 0.42 (321) | 0.9 (306) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 3 (409) | 3 (288) |
| SW124_10 | Upland North | 0.73 (352) | 0.42 (321) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 1 (162) | 3 (288) |
| Pathfinder_02 | Upland West | 0.57 (221) | 0.42 (321) | 0.9 (306) | 0.98 (164) | 3 (448) | 4 (309) | 2 (286) | 3 (266) | 4 (457) | 4 (406) |
| Timber_03 | Lowland South | 0.63 (265) | 0.43 (328) | 0.6 (203) | 0.98 (164) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SWG32_08 | Lowland South | 0.58 (238) | 0.43 (328) | 0.4 (137) | 0.57 (98) | 4 (474) | 5 (396) | 4 (458) | 5 (420) | 3 (409) | 4 (406) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS98.SB_06 | Upland North | 0.88 (453) | 0.44 (330) | 1 (394) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW43_07 | Upland North | 0.67 (293) | 0.44 (330) | 0 (1) | 0.29 (53) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| Blackwell_03 | Upland West | 0.65 (283) | 0.44 (330) | 0.1 (44) | 1 (245) | 2 (294) | 4 (309) | 1 (132) | 2 (150) | 3 (409) | 4 (406) |
| SW124_06 | Upland North | 0.57 (221) | 0.44 (330) | 0 (1) | 0.22 (33) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 3 (409) | 5 (452) |
| SW787_08 | Upland North | 0.73 (352) | 0.44 (330) | 0.2 (89) | 1 (245) | 3 (448) | 5 (396) | 4 (458) | 4 (362) | 2 (322) | 5 (452) |
| ECS.10_08 | Upland East | 0.67 (293) | 0.45 (335) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| Kanlow_06 | Lowland South | 0.68 (318) | 0.45 (335) | 0.9 (306) | 0.97 (155) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| SW129_10 | Upland North | 0.73 (352) | 0.45 (335) | 0.1 (44) | 1 (245) | 1 (101) | 1 (22) | 1 (132) | 1 (75) | 1 (162) | 1 (129) |
| SW65_02 | Upland East | 0.57 (221) | 0.45 (335) | 0.8 (261) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 1 (129) |
| SW109_02 | Upland West | 0.67 (293) | 0.45 (335) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| Dacotah_03 | Upland North | 0.75 (368) | 0.45 (335) | 0.4 (137) | 0.3 (58) | 2 (294) | 5 (396) | 5 (477) | 5 (420) | 0 (1) | 0 (1) |
| SW58_08 | Upland West | 0.7 (328) | 0.46 (341) | 0.6 (203) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW49_06 | Upland North | 0.77 (382) | 0.46 (341) | 0.8 (261) | 0.99 (197) | 0 (1) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW64_07 | Upland East | 0.5 (178) | 0.46 (341) | 1 (394) | 0.97 (155) | 1 (101) | 4 (309) | 1 (132) | 2 (150) | 1 (162) | 4 (406) |
| High.Tide_02 | Lowland North | 0.67 (293) | 0.46 (341) | 0.1 (44) | 0.11 (1) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW49_07 | Upland North | 0.6 (246) | 0.46 (341) | 0.8 (261) | 0.99 (197) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |
| Cave.in.Rock_02 | Upland East | 0.8 (410) | 0.46 (341) | 0.9 (306) | 0.97 (155) | 2 (294) | 4 (309) | 2 (286) | 2 (150) | 3 (409) | 4 (406) |
| SW128_04 | Upland West | 0.63 (265) | 0.47 (347) | 0.9 (306) | 0.98 (164) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW40_10 | Upland North | 0.67 (293) | 0.47 (347) | 0.3 (119) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 1 (129) |
| SW797_02 | Lowland North | 0.58 (238) | 0.47 (347) | 0.9 (306) | 0.95 (139) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| SW110_07 | Upland West | 0.63 (265) | 0.47 (347) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| SW50_04 | Upland West | 0.78 (403) | 0.47 (347) | 0.9 (306) | 0.97 (155) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 1 (162) | 2 (172) |
| SW51_04 | Upland West | 0.67 (293) | 0.47 (347) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 3 (409) | 3 (288) |
| SW129_09 | Upland North | 0.5 (178) | 0.47 (347) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 1 (162) | 2 (172) |
| SW787_04 | Upland North | 0.67 (293) | 0.47 (347) | 0.6 (203) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 1 (129) |
| ECS.2_02 | Upland West | 0.82 (422) | 0.48 (355) | 0.1 (44) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| Pathfinder_10 | Upland West | 0.75 (368) | 0.48 (355) | 0.5 (177) | 0.66 (105) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| WS98.SB_10 | Upland Mix | 0.53 (198) | 0.48 (355) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 3 (409) | 3 (288) |
| SW49_05 | Upland North | 0.63 (265) | 0.48 (355) | 0.4 (137) | 0.74 (113) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| SW58_07 | Upland West | 0.7 (328) | 0.49 (359) | 0.8 (261) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW58_03 | Upland West | 0.8 (410) | 0.49 (359) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 1 (132) | 2 (150) | 2 (322) | 5 (452) |
| ECS.2_03 | Upland West | 0.67 (293) | 0.49 (359) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 2 (322) | 3 (288) |
| SW116_08 | Upland North | 0.77 (382) | 0.49 (359) | 0.9 (306) | 0.99 (197) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 1 (162) | 1 (129) |
| SW102_07 | Upland North | 0.73 (352) | 0.49 (359) | 0.8 (261) | 0.91 (131) | 3 (448) | 5 (396) | 3 (407) | 5 (420) | 2 (322) | 4 (406) |
| SW123_07 | Upland East | 0.8 (410) | 0.5 (364) | 0.2 (89) | 0.91 (131) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| WS98.SB_09 | Upland Mix | 0.7 (328) | 0.5 (364) | 0.5 (177) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| ECS.2_07 | Upland West | 0.93 (467) | 0.5 (364) | 0.5 (177) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW112_03 | Upland West | 0.75 (368) | 0.5 (364) | 0.1 (44) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW31_04 | Upland North | 0.87 (443) | 0.5 (364) | 0.5 (177) | 1 (245) | 2 (294) | 4 (309) | 4 (458) | 4 (362) | 0 (1) | 1 (129) |
| SW64_05 | Upland East | 0.85 (439) | 0.51 (369) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW33_05 | Upland West | 0.65 (283) | 0.51 (369) | 0.2 (89) | 0.51 (91) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW65_09 | Upland North | 0.78 (403) | 0.51 (369) | 0.2 (89) | 0.39 (72) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| WS98.SB_05 | Upland North | 0.65 (283) | 0.51 (369) | 0.9 (306) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 2 (322) | 3 (288) |
| SW109_10 | Upland West | 0.67 (293) | 0.51 (369) | 0.9 (306) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 1 (162) | 2 (172) |
| Carthage_06 | Upland West | 0.85 (439) | 0.51 (369) | 0.7 (236) | 0.91 (131) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 2 (322) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW40_09 | Upland North | 0.8 (410) | 0.51 (369) | 0.3 (119) | 1 (245) | 4 (474) | 5 (396) | 2 (286) | 2 (150) | 4 (457) | 5 (452) |
| SW102_09 | Upland North | 0.7 (328) | 0.52 (376) | 0.2 (89) | 1 (245) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| SW127_04 | Upland West | 0.63 (265) | 0.52 (376) | 0.6 (203) | 0.96 (144) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW116_10 | Upland North | 0.8 (410) | 0.52 (376) | 0.8 (261) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW128_02 | Upland West | 0.82 (422) | 0.52 (376) | 0.2 (89) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW115_05 | Upland North | 0.7 (328) | 0.52 (376) | 0.4 (137) | 0.99 (197) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 0 (1) | 1 (129) |
| ECS.10_01 | Upland East | 0.7 (328) | 0.53 (381) | 0.1 (44) | 0.32 (62) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| WS4U_02 | Upland North | 0.75 (368) | 0.53 (381) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW46_01 | Upland North | 0.58 (238) | 0.53 (381) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 4 (362) | 1 (162) | 2 (172) |
| SW49_04 | Upland North | 0.67 (293) | 0.53 (381) | 0.5 (177) | 0.81 (119) | 1 (101) | 3 (161) | 3 (407) | 3 (266) | 0 (1) | 0 (1) |
| SW124_02 | Upland North | 0.7 (328) | 0.53 (381) | 0.9 (306) | 0.98 (164) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 0 (1) | 2 (172) |
| Blackwell_01 | Upland West | 0.65 (283) | 0.53 (381) | 1 (394) | 1 (245) | 3 (448) | 5 (396) | 3 (407) | 4 (362) | 4 (457) | 5 (452) |
| SW112_02 | Upland West | 0.78 (403) | 0.54 (387) | 0.4 (137) | 0.93 (137) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| Dacotah_02 | Upland North | 0.77 (382) | 0.54 (387) | 0.5 (177) | 1 (245) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 2 (322) | 3 (288) |
| SW129_08 | Upland North | 0.83 (432) | 0.55 (389) | 0.9 (306) | 0.98 (164) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW38_03 | Upland West | 0.68 (318) | 0.55 (389) | 0.9 (306) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 1 (129) |
| Sunburst_09 | Upland West | 0.73 (352) | 0.55 (389) | 0 (1) | 0.14 (5) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 3 (409) | 4 (406) |
| SW129_05 | Upland North | 0.9 (456) | 0.55 (389) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 1 (162) | 2 (172) |
| SW109_05 | Upland West | 0.77 (382) | 0.56 (393) | 1 (394) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW112_04 | Upland West | 0.77 (382) | 0.56 (393) | 0.4 (137) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 3 (409) | 3 (288) |
| SW65_10 | Upland North | 0.82 (422) | 0.56 (393) | 0.6 (203) | 1 (245) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 2 (322) | 3 (288) |
| SW793_06 | Lowland North | 0.82 (422) | 0.57 (396) | 0.1 (44) | 0.31 (60) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW796_10 | Lowland North | 0.73 (352) | 0.57 (396) | 0.3 (119) | 0.44 (81) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |
| ECS.2_06 | Upland West | 0.77 (382) | 0.57 (396) | 0.6 (203) | 0.73 (112) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 1 (129) |
| Kanlow_02 | Lowland South | 0.78 (403) | 0.57 (396) | 0.9 (306) | 0.99 (197) | 2 (294) | 3 (161) | 0 (1) | 1 (75) | 3 (409) | 3 (288) |
| SW124_05 | Upland North | 0.67 (293) | 0.57 (396) | 0.8 (261) | 0.96 (144) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW128_01 | Upland West | 0.7 (328) | 0.58 (401) | 0.8 (261) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 3 (409) | 3 (288) |
| SW110_09 | Upland West | 0.73 (352) | 0.58 (401) | 0.8 (261) | 1 (245) | 1 (101) | 2 (54) | 0 (1) | 0 (1) | 2 (322) | 2 (172) |
| SW58_02 | Upland West | 0.68 (318) | 0.58 (401) | 0.9 (306) | 0.99 (197) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| SW110_08 | Upland West | 0.5 (178) | 0.58 (401) | 0.9 (306) | 0.98 (164) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ECS.12_07 | Upland West | 0.77 (382) | 0.58 (401) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| Dacotah_05 | Upland North | 0.7 (328) | 0.58 (401) | 0.2 (89) | 0.56 (95) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 1 (129) |
| SW786_07 | Upland North | 0.92 (465) | 0.58 (401) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW49_03 | Upland North | 0.8 (410) | 0.58 (401) | 0.6 (203) | 0.85 (123) | 3 (448) | 5 (396) | 3 (407) | 3 (266) | 3 (409) | 5 (452) |
| Kanlow_08 | Lowland South | 0.77 (382) | 0.59 (409) | 0.1 (44) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 1 (129) |
| SW786_01 | Upland North | 0.93 (467) | 0.59 (409) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 1 (162) | 2 (172) |
| SW110_05 | Upland West | 0.75 (368) | 0.59 (409) | 0.9 (306) | 0.95 (139) | 2 (294) | 3 (161) | 1 (132) | 1 (75) | 3 (409) | 3 (288) |
| SW781_06 | Lowland North | 0.67 (293) | 0.59 (409) | 0.2 (89) | 0.99 (197) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 3 (288) |
| SW58_09 | Upland West | 0.73 (352) | 0.6 (413) | 0.7 (236) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 1 (75) | 2 (322) | 3 (288) |
| SW110_03 | Upland West | 0.77 (382) | 0.6 (413) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 1 (75) | 2 (322) | 3 (288) |
| SW122_01 | Upland West | 0.85 (439) | 0.6 (413) | 0.6 (203) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 1 (129) |
| Cave.in.Rock_10 | Upland East | 0.82 (422) | 0.6 (413) | 0.8 (261) | 1 (245) | 2 (294) | 3 (161) | 0 (1) | 1 (75) | 3 (409) | 3 (288) |
| SW51_05 | Upland West | 0.82 (422) | 0.61 (417) | 1 (394) | 1 (245) | 1 (101) | 4 (309) | 1 (132) | 2 (150) | 1 (162) | 4 (406) |
| SW115_04 | Upland North | 0.75 (368) | 0.62 (418) | 0 (1) | 0.2 (22) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| Pathfinder_08 | Upland West | 0.7 (328) | 0.62 (418) | 0.6 (203) | 0.99 (197) | 2 (294) | 3 (161) | 2 (286) | 2 (150) | 2 (322) | 3 (288) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS4U_06 | Upland North | 0.8 (410) | 0.62 (418) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 3 (288) |
| SW109_04 | Upland West | 0.9 (456) | 0.62 (418) | 0.4 (137) | 0.68 (107) | 3 (448) | 5 (396) | 2 (286) | 3 (266) | 5 (473) | 5 (452) |
| SW786_09 | Upland North | 0.8 (410) | 0.63 (422) | 0.7 (236) | 0.98 (164) | 1 (101) | 3 (161) | 1 (132) | 3 (266) | 1 (162) | 2 (172) |
| SW129_02 | Upland North | 0.83 (432) | 0.63 (422) | 0.5 (177) | 0.98 (164) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 0 (1) |
| SW46_04 | Upland North | 0.65 (283) | 0.63 (422) | 0.4 (137) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| Sunburst_02 | Upland West | 0.9 (456) | 0.63 (422) | 0.8 (261) | 1 (245) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| SW51_06 | Upland West | 0.87 (443) | 0.63 (422) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 2 (286) | 3 (266) | 0 (1) | 1 (129) |
| ECS.12_02 | Upland West | 0.82 (422) | 0.64 (427) | 0.5 (177) | 0.96 (144) | 1 (101) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW110_06 | Upland West | 0.7 (328) | 0.64 (427) | 0.9 (306) | 0.96 (144) | 2 (294) | 3 (161) | 1 (132) | 1 (75) | 3 (409) | 3 (288) |
| Pathfinder_09 | Upland West | 0.83 (432) | 0.65 (429) | 0.7 (236) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| Timber_04 | Lowland South | 0.9 (456) | 0.65 (429) | 0.6 (203) | 0.99 (197) | 3 (448) | 3 (161) | 3 (407) | 3 (266) | 2 (322) | 3 (288) |
| SW51_09 | Upland West | 0.77 (382) | 0.66 (431) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| WS4U_05 | Upland North | 0.7 (328) | 0.66 (431) | 0.3 (119) | 0.88 (127) | 1 (101) | 5 (396) | 2 (286) | 5 (420) | 0 (1) | 0 (1) |
| SW31_07 | Upland North | 0.65 (283) | 0.66 (431) | 0.9 (306) | 0.96 (144) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| SW102_03 | Upland North | 0.77 (382) | 0.66 (431) | 0.4 (137) | 1 (245) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 4 (406) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW116_07 | Upland North | 0.87 (443) | 0.67 (435) | 0.7 (236) | 0.69 (109) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 0 (1) | 1 (129) |
| SW116_09 | Upland North | 0.82 (422) | 0.67 (435) | 0.9 (306) | 1 (245) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 2 (322) | 3 (288) |
| SW33_06 | Upland West | 0.87 (443) | 0.68 (437) | 0.7 (236) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 1 (129) |
| SW31_06 | Upland North | 0.57 (221) | 0.69 (438) | 1 (394) | 1 (245) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) | 0 (1) |
| SW46_08 | Upland North | 0.87 (443) | 0.69 (438) | 1 (394) | 1 (245) | 2 (294) | 3 (161) | 3 (407) | 3 (266) | 1 (162) | 3 (288) |
| SW809_01 | Upland East | 0.8 (410) | 0.7 (440) | 0.8 (261) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 2 (150) | 1 (162) | 3 (288) |
| SW786_06 | Upland North | 0.87 (443) | 0.7 (440) | 0.8 (261) | 0.91 (131) | 2 (294) | 5 (396) | 4 (458) | 5 (420) | 2 (322) | 3 (288) |
| SW112_10 | Upland West | 0.8 (410) | 0.71 (442) | 0.6 (203) | 0.98 (164) | 0 (1) | 2 (54) | 0 (1) | 1 (75) | 1 (162) | 2 (172) |
| SW122_04 | Upland West | 0.87 (443) | 0.71 (442) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW115_02 | Upland North | 0.83 (432) | 0.71 (442) | 0.9 (306) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |
| Pathfinder_01 | Upland West | 0.8 (410) | 0.72 (445) | 1 (394) | 0.99 (197) | 1 (101) | 5 (396) | 0 (1) | 0 (1) | 4 (457) | 5 (452) |
| Cave.in.Rock_08 | Upland East | 0.75 (368) | 0.72 (445) | 0.4 (137) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 1 (129) |
| Cave.in.Rock_03 | Upland East | 0.82 (422) | 0.72 (445) | 0.9 (306) | 0.88 (127) | 2 (294) | 3 (161) | 2 (286) | 3 (266) | 3 (409) | 3 (288) |
| SW102_08 | Upland North | 0.77 (382) | 0.73 (448) | 1 (394) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW124_07 | Upland North | 0.93 (467) | 0.73 (448) | 0.6 (203) | 1 (245) | 1 (101) | 2 (54) | 2 (286) | 2 (150) | 0 (1) | 0 (1) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SW109_06 | Upland West | 0.73 (352) | 0.73 (448) | 0.8 (261) | 1 (245) | 2 (294) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| Blackwell_08 | Upland West | 0.9 (456) | 0.74 (451) | 1 (394) | 1 (245) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| Kanlow_07 | Lowland South | 0.93 (467) | 0.74 (451) | 0.2 (89) | 0.4 (74) | 2 (294) | 3 (161) | 1 (132) | 3 (266) | 2 (322) | 3 (288) |
| SW115_01 | Upland North | 0.97 (475) | 0.75 (453) | 0.8 (261) | 0.99 (197) | 0 (1) | 2 (54) | 0 (1) | 0 (1) | 1 (162) | 2 (172) |
| Sunburst_01 | Upland West | 0.93 (467) | 0.75 (453) | 1 (394) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 1 (75) | 0 (1) | 0 (1) |
| SW790_07 | Lowland/Upland Mix | 0.85 (439) | 0.75 (453) | 0.9 (306) | 0.99 (197) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 1 (162) | 3 (288) |
| SW31_05 | Upland North | 0.83 (432) | 0.75 (453) | 1 (394) | 0.98 (164) | 2 (294) | 4 (309) | 3 (407) | 4 (362) | 0 (1) | 0 (1) |
| WS4U_07 | Upland North | 0.87 (443) | 0.75 (453) | 1 (394) | 1 (245) | 3 (448) | 5 (396) | 4 (458) | 5 (420) | 1 (162) | 3 (288) |
| SW129_06 | Upland North | 0.93 (467) | 0.76 (458) | 0 (1) | 0.16 (11) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW51_08 | Upland West | 0.73 (352) | 0.78 (459) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 3 (288) |
| ECS.10_09 | Upland East | 0.77 (382) | 0.79 (460) | 0.7 (236) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW124_09 | Upland North | 0.83 (432) | 0.79 (460) | 0.7 (236) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 4 (362) | 1 (162) | 2 (172) |
| Sunburst_06 | Upland West | 0.77 (382) | 0.81 (462) | 0.9 (306) | 0.99 (197) | 1 (101) | 4 (309) | 0 (1) | 0 (1) | 3 (409) | 4 (406) |
| Sunburst_08 | Upland West | 0.93 (467) | 0.81 (462) | 1 (394) | 1 (245) | 3 (448) | 5 (396) | 2 (286) | 3 (266) | 4 (457) | 5 (452) |
| SW129_04 | Upland North | 0.77 (382) | 0.82 (464) | 0.7 (236) | 1 (245) | 0 (1) | 1 (22) | 0 (1) | 0 (1) | 0 (1) | 1 (129) |

| Genotypes | Ecotypes | DTVI | DTIA | DSVI | DSIA | mean both fields | MTL | mean NY | MNY | Mean PA | MPA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sunburst_04 | Upland West | 0.97 (475) | 0.82 (464) | 0.9 (306) | 0.99 (197) | 2 (294) | 2 (54) | 1 (132) | 2 (150) | 2 (322) | 2 (172) |
| SW50_02 | Upland West | 0.9 (456) | 0.83 (466) | 0.9 (306) | 0.99 (197) | 1 (101) | 2 (54) | 1 (132) | 2 (150) | 1 (162) | 2 (172) |
| SW809_03 | Upland East | 0.88 (453) | 0.83 (466) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 2 (322) | 4 (406) |
| Pathfinder_04 | Upland West | 0.92 (465) | 0.83 (466) | 0.9 (306) | 1 (245) | 2 (294) | 4 (309) | 2 (286) | 3 (266) | 3 (409) | 4 (406) |
| SW127_05 | Upland West | 0.93 (467) | 0.84 (469) | 0.5 (177) | 1 (245) | 1 (101) | 3 (161) | 0 (1) | 0 (1) | 2 (322) | 3 (288) |
| SW102_10 | Upland North | 0.87 (443) | 0.84 (469) | 0.6 (203) | 0.99 (197) | 3 (448) | 5 (396) | 4 (458) | 5 (420) | 2 (322) | 2 (172) |
| SW63_04 | Upland North | 0.97 (475) | 0.85 (471) | 0.9 (306) | 1 (245) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW127_03 | Upland West | 0.77 (382) | 0.86 (472) | 1 (394) | 0.98 (164) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 2 (322) | 3 (288) |
| SW64_10 | Upland East | 0.9 (456) | 0.87 (473) | 0.4 (137) | 0.44 (81) | 1 (101) | 4 (309) | 2 (286) | 4 (362) | 0 (1) | 0 (1) |
| SW64_01 | Upland East | 0.9 (456) | 0.87 (473) | 0.5 (177) | 0.49 (89) | 1 (101) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |
| SW63_05 | Upland North | 1 (479) | 0.88 (475) | 1 (394) | 1 (245) | 2 (294) | 5 (396) | 2 (286) | 5 (420) | 3 (409) | 4 (406) |
| SW809_07 | Upland East | 0.9 (456) | 0.89 (476) | 1 (394) | 1 (245) | 1 (101) | 3 (161) | 1 (132) | 2 (150) | 1 (162) | 3 (288) |
| SW64_04 | Upland East | 0.87 (443) | 0.9 (477) | 0 (1) | 0.13 (3) | 0 (1) | 2 (54) | 1 (132) | 2 (150) | 0 (1) | 0 (1) |
| SW64_02 | Upland East | 0.77 (382) | 0.92 (478) | 1 (394) | 1 (245) | 0 (1) | 3 (161) | 1 (132) | 3 (266) | 0 (1) | 0 (1) |
| SW114_04 | Upland North | 0.97 (475) | 0.95 (479) | 1 (394) | 0.95 (139) | 2 (294) | 5 (396) | 3 (407) | 5 (420) | 0 (1) | 0 (1) |

Table II.3 Phenotype variance explained (PVE) by each significant SNP markers in each subgroup for each trait.

| Subgroups | Trait | SNP | PVE (adjusted PVE) |
|---|---|---|---|
| 479 | DTIA | snp151084 | 0.01 (-0.2) |
| | | snp680175 | 0 (-0.21) |
| | | snp359167 | 0.09 (-0.12) |
| | | snp933838 | 0.44 (0.24) |
| | | snp938364 | 0.36 (0.15) |
| | | snp465249 | 0 (-0.21) |
| | | snp469523 | 0.66 (0.45) |
| | | snp1003871 | 0.03 (-0.18) |
| | DSIA | snp151084 | 3.97 (3.77) |
| | | snp680175 | 3.61 (3.41) |
| | | snp359167 | 3.09 (2.89) |
| | | snp933838 | 2.86 (2.65) |
| | | snp938364 | 2.02 (1.81) |
| | | snp465249 | 4.35 (4.15) |
| | | snp469523 | 3.01 (2.81) |
| | | snp1003871 | 3.14 (2.93) |
| | MNY | snp151084 | 1.41 (1.2) |
| | | snp680175 | 0.89 (0.69) |
| | | snp359167 | 0.3 (0.09) |
| | | snp933838 | 1.23 (1.03) |
| | | snp938364 | 1.77 (1.56) |
| | | snp465249 | 3.69 (3.49) |
| | | snp469523 | 0.95 (0.75) |
| | | snp1003871 | 2.42 (2.21) |
| 4X | MNY | snp316732 | 0.31 (-0.06) |
| | | snp444058 | 1.56 (1.19) |
| low | DTIA | snp594527 | 1.2 (0.46) |
| | | snp197006 | 0.03 (-0.72) |
| | | snp732235 | 1.07 (0.33) |
| | | snp791318 | 0.1 (-0.64) |
| | | snp791375 | 0.2 (-0.54) |
| | DSIA | snp594527 | 6.77 (6.07) |
| | | snp197006 | 3.59 (2.87) |
| | | snp732235 | 0.06 (-0.69) |
| | | snp791318 | 0.38 (-0.36) |
| | | snp791375 | 1.24 (0.5) |

| Subgroups | Trait | SNP | PVE (adjusted PVE) |
|---|---|---|---|
| | MNY | snp594527 | 0.57 (-0.17) |
| | | snp197006 | 4.58 (3.86) |
| | | snp732235 | 2.92 (2.19) |
| | | snp791318 | 1.29 (0.55) |
| | | snp791375 | 0.63 (-0.11) |
| | MPA | snp594527 | 11 (10.34) |
| | | snp197006 | 8.67 (7.99) |
| | | snp732235 | 15.71 (15.08) |
| | | snp791318 | 17.08 (16.46) |
| | | snp791375 | 11.65 (11) |
| up | DTVI | snp411929 | 6.31 (6.04) |
| | | snp411995 | 6.38 (6.11) |
| | | snp412960 | 5.47 (5.21) |
| | MNY | snp411929 | 0 (-0.28) |
| | | snp411995 | 0.1 (-0.18) |
| | | snp412960 | 0 (-0.28) |

Abbreviation: BLUPs of severity from the leaf detachment via vision (DTVI), BLUPs of severity from the leaf detachment via image analysis (DTIA), BLUPs of severity from the leaf disk assay via image analysis (DSIA), highest score of BLS in NY (MNY), highest score of BLS in PA (MPA).